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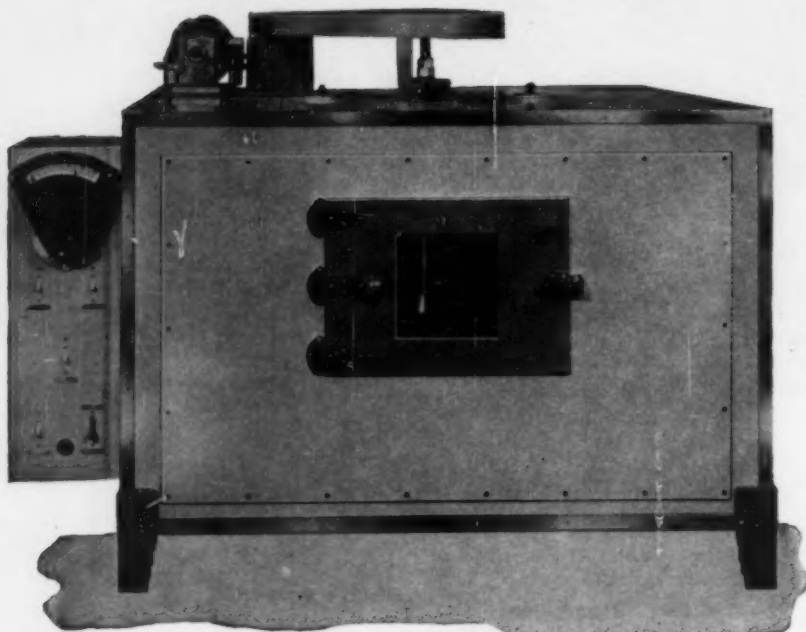
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No. 1

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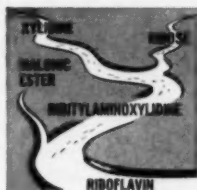
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# CEREAL CHEMISTRY

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No. 1

## STUDIES ON THE BREW PROCESS OF BREAD MANUFACTURE: THE EFFECT OF SUGAR AND OTHER NUTRIENTS ON BAKING QUALITY AND YEAST PROPERTIES<sup>1</sup>

J. W. LEE AND W. F. GEDDES

### ABSTRACT

Yeast extract (the water-soluble fraction of autolyzed yeast) was a more satisfactory nutrient in brews than was casein hydrolysate, an aqueous extract of flour or various synthetic media. These brews were made by a basic formula containing sodium chloride, yeast, and sucrose in succinate buffer (0.02M, pH 5.5). Yeast which had undergone brew fermentation in the presence of yeast extract had an increased rate of gas production and an increased nitrogen content. The presence of fermentable sugar in the brew was essential to obtain the beneficial effect of yeast extract. Yeast extract did not affect the total yeast count, mean cell diameter, or population of budding cells.

The gas production and baking quality of brews containing glucose or sucrose were equally satisfactory. When used as the only sugar in the brew, maltose remained essentially unfermented after 6 hours at 37°C. If, however, a maltose-glucose mixture (9:1) was used in the brew, gas production and baking quality were greatly improved. Regardless of the sugar employed in brew preparation, the maltose content of the dough increased throughout the 90-minute fermentation period at 30°C. The maltose content of bread made by the brew process was much higher than that found by other workers in bread made by the sponge and dough procedure.

In recent years there has been considerable interest in the brew process of bread manufacture as a replacement for the sponge in the customary sponge and dough procedure (2,8,12,13,14,15,17,20; footnote 2). The brew process, also known as the stable-ferment or pre-ferment method, is considered as a likely part of any continuous breadmaking procedure; in fact, it is at present employed in at least one continuous process (4). The essential components of a brew are yeast, sugar, and

<sup>1</sup> Manuscript received December 16, 1957. Contribution from the Department of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota. Paper No. 3882 of the Scientific Journal Series, Minnesota Agricultural Experiment Station. The data in this paper are taken from a thesis presented to the Graduate School of the University of Minnesota by John W. Lee in partial fulfillment of the requirements for the Ph.D. degree, August, 1957.

other nutrients, and water in a buffered system. A buffer, such as nonfat dry milk or calcium carbonate, is essential to maintain the pH between 4.5 and 5.5; otherwise, losses in yeast activity occur (8,13,14; footnote 2). Sodium chloride and other optional ingredients like oxidizing agents, enrichment substances, malt, and enzyme preparations are also frequently added. The brew is fermented under controlled conditions for 4 to 6 hours at 25° to 38°C. before being added to the flour to form a dough. The dough is then handled in the same manner as one made by the sponge and dough procedure.

McLaren *et al.*<sup>2</sup> have studied a number of the changes which occur in fermenting brews prepared by the "Stable-Ferment Process" of the American Dry Milk Institute (ADMI) (2), which employs nonfat dry milk as the buffer. They did not find any change in yeast population during the 6-hour fermentation period but there was evidence of an increase in cell size. The titratable acidity and lactic acid increased during fermentation, whereas the pH decreased. Both alcohol and gas production were complete after 2 hours. Sugar was essential to produce brews of satisfactory baking quality.

Johnson *et al.* (9) studied gas production, sugar utilization, pH changes, and acid production in fermenting brews. They observed that milk increased the rate of carbon dioxide production and prevented excessive decreases in pH. Milk, however, did not greatly influence the total acidity of brews. Carroll, Miller, and Johnson (5) investigated the activity of fungal alpha-amylase, protease, and malted wheat flour in brews. If no buffer system was included, the activity of the enzymes decreased with time because of the marked decrease in pH. In adequately buffered brews, however, there was little or no loss of enzymatic activity; similar findings were made by Maselli (13,14), using calcium carbonate as the buffer. Robinson *et al.* (18) and McLaren *et al.* (see footnote 2) observed a sharp initial decline in bacterial population upon dough mixing. The former authors suggested that this decline was caused by an antibiotic elaborated by the yeast.

This paper reports the development of a laboratory-scale procedure for making bread by the brew process, employing a succinate buffer to provide a completely soluble and chemically defined system except for the yeast. This procedure was employed in studying the changes in baking quality and yeast properties which occurred when various sources of nutrients were used for brew preparation. The relative rates of utilization of individual sugars in brews and in doughs made from them were also studied.

<sup>2</sup> McLaren, L. M., De Brower, C., Choi, R. P., Kocua, A. F., and Remaley, R. J. A new ferment process for making bread. Paper presented at 39th annual meeting, AACC, Denver, 1954.

### Development of Laboratory-Scale Procedure for Making Bread by the Brew Process Employing Succinate Buffer

As a basis for the development of a soluble brew medium, the brew formula of the American Dry Milk Institute (2) was reduced to a laboratory scale to permit baking tests employing 100 g. of flour per loaf.

*Preparation of Brews.* The laboratory formula for the ADMI brew, expressed in grams, was: water, 100.0; yeast, 6.25; sucrose, 6.25; sodium chloride, 3.15; nonfat dry milk, 18.75. To obtain a soluble medium, two nutrient-buffer systems were tried; namely, 0.02M sodium citrate/citric acid buffer, pH 6.2, and 0.02M sodium succinate/succinic acid buffer, pH 5.5. To 100 ml. of each of these buffers, 80 mg. ammonium sulfate, 3 mg. calcium chloride, and 50 mg. dipotassium phosphate were added. These solutions replaced the water and nonfat dry milk in the ADMI brew formula. The brews were fermented for 6.0 hours at 37°C. in Erlenmeyer flasks (250-ml.) with indentations in the base. They were shaken horizontally at a sufficiently rapid rate to keep the yeast in suspension.

*Baking Formulas and Procedures.* The following dough formula was employed in baking tests on brews:

Ingredient	Quantity
	g
Flour <sup>a</sup>	100.0
Sodium chloride	1.0
Compressed yeast	1.0
Shortening (hydrogenated vegetable)	2.0
Nonfat dry milk <sup>b</sup> (prepared for breadmaking)	6.0
Sucrose	5.0
Potassium bromate	0.001
Brew	36.0
Water	as required

<sup>a</sup> The flour used was a Southwestern Baker's Patent (protein 11.3%, ash 0.47%, on a 14% moisture basis).

<sup>b</sup> Nonfat dry milk was not added at the dough stage in tests on the ADMI brew.

The doughs were mixed for 3.5 minutes in a Swanson mixer fitted with a McDuffee bowl. The water temperature was adjusted so that the doughs left the mixer at  $30^{\circ} \pm 0.5^{\circ}\text{C}$ . The dough was fermented for 30 minutes at 30°C. before being scaled to 150 g., molded, and placed in a baking pan. The dough was proofed at 30°C. to a fixed height, then baked for 25 minutes at 232°C. Loaf volume was measured by displacement of rape seed, and an arbitrary score (maximum 6) for grain and texture was assigned.

*Results of Baking Tests.* The comparative baking qualities and pH changes for the ADMI "citrate" and "succinate" brews follow:

Brew Type	pH of Brew		Loaf Volume*	Proof Time	Grain and Texture (Max. 6)
	Initial	Final			
			cc	minutes	
ADMI	5.8	5.1	640	65	4
"Citrate"	6.0	5.2	650	70	4
"Succinate"	5.8	5.0	655	70	4

\* Mean of four loaves.

The pH changes and loaf volumes obtained with the three brews were very similar. The succinate buffer was selected for further use, as citrate was considered more likely to "complex" some of the metallic ions necessary for the nutrition of the yeast. The succinate buffer was used without the addition of the mineral salts.

### Materials and Methods

*Preparation of Brews.* In all subsequent studies the basic formula for brews was as follows:

	g
Compressed yeast	6.25
Sodium chloride	3.15
Sugar*	6.25
0.02M sodium succinate/ succinic acid buffer, pH 5.5, 100 ml.	

\* Unless otherwise indicated, sugar refers to sucrose.

These brews were fermented in the manner previously described.

*Baking Tests.* The brews were used in baking tests following the formula and procedure described in the preliminary experiments, except that the doughs were proofed to time (60 minutes). A record was made of the height of the dough above the top of the pan immediately prior to baking.

All baking tests were carried out in duplicate on each of two successive days, and the values which are recorded therefore represent the means of four loaves.

To obtain some measure of statistically significant differences between loaf volumes, an experiment was made in which 72 loaves representing 36 different treatments were baked on each of two successive days. The loaf volumes obtained in this experiment are summarized in Table III, and covered the range encountered in most other trials. Taking the variance for the interaction of sugar content and yeast level as a measure of error, the standard error (single determination) is 16.5 cc. Since there were four replications for each treatment, the least significant difference between two sample means equals  $2 \times 16.5$

$\sqrt{2}/\sqrt{4}$  or 23.3 cc. Accordingly, in all experiments involving measurement of loaf volume, sample means were considered significantly different if they varied by more than 24 cc.

*Gas Production in Brews.* A 5-ml. aliquot of the freshly mixed brew was pipetted into a 25-ml. Erlenmeyer flask which, in turn, was placed in a pressuremeter cup of the type described in *Cereal Laboratory Methods* (1). The bowl of the pressuremeter was completely immersed in a water bath maintained at 37°C. The pressuremeter and its contents were agitated at frequent intervals and pressure readings were made at appropriate intervals.

*Sugar Determinations in Brews, Doughs, and Bread.* The procedure for the removal and chromatographic separation of the individual sugars present in fermenting doughs and in bread was similar to that described by Koch, Geddes, and Smith (10,11). The preparation of the sample and chromatography of the sugars were conducted exactly as described by these authors. The method of location of the sugars on the chromatograms was, however, modified in the following manner:

Two 0.25-in. strips were cut longitudinally from the developed chromatogram 2.5 in. from the edge. These strips were sprayed with ammoniacal silver nitrate (equal volumes of 2N silver nitrate solution and ammonium hydroxide, sp. gr. 0.880) and heated at 130°C. until the spots developed. These strips were reassembled into the chromatograms and the location of the sugars marked on the unsprayed portion.<sup>3</sup> The portions of the chromatogram containing the individual sugars were cut out and eluted with a known volume of water as described by Koch, Geddes, and Smith (10,11). Sugar determinations on the eluates were carried out by the phenolsulfuric acid method of Dubois *et al.* (6), allowance being made for the quantity of sugar on the strips used for location of the sugars. The amount of sugar on these strips was determined from the relative widths of the strips and the starting line on the chromatogram.

Brews were prepared for sugar analyses in the following manner: To 10 ml. of boiling absolute ethanol was added a 10-ml. aliquot of the brew. The mixture was boiled for 1 minute, then cooled to room temperature before being made to a volume of 25 ml. This solution was centrifuged and 20 ml. of the supernatant fluid dialyzed against 50 ml. of 40% ethanol for 36 hours at 5°C. A 25-ml. aliquot of the dial-

<sup>3</sup> This method of locating the position of the sugars on the developed chromatogram proved to be more reliable than the procedure used by Koch, Geddes, and Smith (10,11), which involved the use of separate spots on each side of the chromatogram to locate the sugars on the unsprayed center portion. The sugars at these "spots" frequently did not migrate at the same rate as samples of the same sugars streaked across the central portion of the chromatogram. While the accuracy of the method used in the present investigation is dependent upon even distribution of the sugar along the starting line of the chromatogram, this difficulty was more than offset by the possible error in the former method.

ysate was deionized with mixed Amberlite MB-1 resin (Rohm and Haas Co., Philadelphia, Pa.), and evaporated to dryness *in vacuo* at approximately 50°C. The residue was taken up in a known volume of water; the sugars present were separated chromatographically and their concentrations determined by the same methods as were used for dough and bread.

Other procedures which were employed in only single experiments are described along with the results in succeeding sections of this paper.

*Effect of Various Sources of Yeast Nutrients on the Baking Quality of Brews.* An attempt was made to improve the baking quality of "succinate" brews by adding yeast nutrients. Three sources of nutrients were investigated initially; Difco yeast extract (Difco Laboratories, Detroit, Michigan), which is the water-soluble portion of autolyzed yeast, an aqueous extract of flour, and acid-hydrolyzed casein (Difco "casamino acids"). The flour extract was prepared as follows:

Flour (300 g.) was shaken with 1500 ml. water at room temperature for 30 minutes. The suspension was centrifuged at 2000 r.p.m. for 15 minutes and the supernatant decanted and freeze-dried.

The effects of various levels of these three sources of nutrients on the baking quality of "succinate" brews are given in Table I.

TABLE I  
THE EFFECT OF YEAST EXTRACT, FLOUR EXTRACT, AND  
CASEIN HYDROLYSATES ON THE BAKING QUALITY OF BREWS

EXTRACT ADDITION PER 100 ML. BREW	LOAF VOLUME*	PROOF HEIGHT	GRAIN AND TEXTURE (MAXIMUM 6)
<i>g</i>	<i>cc</i>	<i>mm</i>	
No addition	640	6	3
Yeast extract, 0.1	640	6	3
Yeast extract, 0.2	650	7	4
Yeast extract, 0.5	660	6	4
Yeast extract, 1.0	700	10	5
Yeast extract, 2.0	695	8	5
Yeast extract, 5.0	675	4	5
Flour extract, 1.0	620	0	4
Flour extract, 5.0	610	-2	4
Casein hydrolysate, 0.5	660	7	4
Casein hydrolysate, 1.0	650	5	4
Casein hydrolysate, 2.0	660	6	4

\* Least significant difference, 24 cc.

The aqueous flour extract, at both levels used, depressed loaf volume although it caused a slight improvement in grain and texture. Yeast extract, on the other hand, improved the baking quality of brews, the optimum level being in the 1 to 2% range. Casein hydrolysate, while giving some improvement, was not as effective as yeast extract.

*Effect of Various Synthetic Media on the Baking Quality of Brews.* Until the work of Atkin, Schultz, and Frey (3) in 1945, no investigator had succeeded in obtaining a synthetic medium in which yeast would ferment glucose as rapidly as it would in dough. Other media like that of Olson and Johnson (16) have been developed for the growth of yeast. Some of these synthetic media were compared with yeast extract as sources of nutrients in brew preparation.

Brews (ASF) made with the medium of Atkin, Schultz, and Frey (3) were prepared as follows:

<i>Ingredient</i>	<i>g</i>
Monosodium phosphate ( $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ )	0.3
Magnesium sulfate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ )	0.2
Potassium chloride	0.08
L-asparagine	1.00
Thiamine hydrochloride	0.0004
Pyridoxine hydrochloride	0.0004
Nicotinic acid	0.004

These quantities of nutrients were added to 100 ml. of 0.02M succinate buffer, pH 5.5, containing 3.15 g. sodium chloride, 6.25 g. sucrose, and 6.25 g. yeast to make the "full-strength ASF brew." In other trials one-half and one-quarter quantities of the nutrients were added to full quantities of buffer, sodium chloride, sucrose, and yeast to make "half-strength ASF" and "quarter-strength ASF" brews, respectively. These brews were fermented at 37°C. in the usual way. Difco yeast extract (1%) was added to "full-strength" and "quarter-strength ASF" brews to determine whether any further response in baking quality could be obtained. The results of baking tests on these brews are given in Table II.

The "full-strength ASF" medium in the brew caused an improve-

TABLE II  
THE EFFECT OF SYNTHETIC MEDIA IN BREWS

TREATMENT	LOAF VOLUME <sup>a</sup>	PROOF HEIGHT	GRAIN AND TEXTURE (MAXIMUM 6)
	cc	mm	
No addition	635	3	3
Yeast extract (YE) 1%	700	10	5
ASF <sup>b</sup> full strength	665	3	5
Half strength	645	4	4
Quarter strength	630	5	4
Full strength + 1% YE	685	8	5
Quarter strength + 1% YE	695	10	5

<sup>a</sup> Least significant difference 24 cc.

<sup>b</sup> ASF = medium of Atkin, Schultz, and Frey (3).

ment in baking quality, but the increase in loaf volume was not as great as that obtained with 1% yeast extract alone. The addition of 1% yeast extract to the ASF media caused a further increase in loaf volume. Other synthetic media including that of Olson and Johnson (16) were incorporated into brews, but none were as effective as that of Atkin, Schultz, and Frey (3).

*Combined Effect of Yeast Extract and Sucrose on the Baking Quality of Brews.* To determine whether any additional benefit could be obtained from yeast extract by varying the sugar level, an experiment of factorial design was conducted. "Succinate" brews containing 0, 2, 4, 6, 8, and 10% sucrose and 0, 0.1, 0.5, 1.0, 2.0, and 5.0% yeast extract were prepared and subjected to baking tests. Samples involving each treatment were baked in duplicate and the whole procedure was repeated on the succeeding day. In all, 144 loaves were baked, so that the mean value for each treatment was obtained from four replications. The loaf volumes and proof heights obtained in this series of trials are given in Table III. A variance analysis of the loaf-volume data summarized in Table III follows in Table IV.

TABLE III  
INFLUENCE OF SUCROSE AND YEAST EXTRACT IN BREWS  
ON LOAF VOLUME AND PROOF HEIGHT

YEAST EXTRACT	SUCROSE CONTENT (FLOUR BASIS), PERCENT OF MEAN					
	0	2	4	6	8	10
	Loaf volume, cc.					
0.0	565	615	625	640	625	610
0.1	580	600	635	640	650	635
0.5	590	630	665	660	655	640
1.0	600	650	685	700	710	680
2.0	615	675	675	695	700	650
5.0	590	610	650	675	690	630
Mean	588	630	655	666	671	638
	Proof height, mm.					
0.0	—1	5	6	6	5	2
0.1	0	4	7	7	7	2
0.5	0	4	8	6	7	5
1.0	2	6	9	10	9	8
2.0	2	7	7	8	8	5
5.0	0	1	1	4	4	0

The mean loaf volumes significantly increased as the sugar concentrations rose from 0 to 8%, but at the 10% level the mean loaf volume was lower than at the 8% level. The effect of the concentration of yeast extract was highly significant, with levels of 1 or 2% giving maximum loaf volumes. The variance analysis shows a highly signifi-

TABLE IV  
VARIANCE ANALYSIS OF THE LOAF-VOLUME DATA SUMMARIZED IN TABLE III

VARIATION DUE TO:	DEGREES OF FREEDOM	MEAN SQUARES
Days (D)	1	10764**
Treatments (T)	35	5565**
Levels of sugar (S)	5	22445**
Levels of yeast extract (Y)	5	15140**
Interaction S $\times$ Y	25	273**
Interaction D $\times$ T	35	85
Duplicates (error)	72	123
Total	143	1520

cant interaction between the percentages of sugar and yeast extract.

*Brew Fractionation Studies.* To determine changes which might occur in the baking quality of the yeast and in the baking quality of the medium during brew fermentation, a separation and reconstitution of these components was effected as follows:

A 100-ml. portion of "succinate" brew containing 1% yeast extract was centrifuged at 2000 r.p.m. (International Centrifuge, size 2) for 5 minutes. The supernatant fluid was separated from the sedimented yeast cells and the latter washed twice with water. To the supernatant was added an amount of fresh yeast equivalent to that originally added to the brew. The sedimented yeast cells were suspended in a volume of fresh medium equal to the volume of supernatant separated. This medium was the same as that used for the preparation of the original brew except that no sugar was added. In addition, a further system was prepared containing fresh yeast and fresh medium. Samples of the original brew and the three reconstituted samples were incorporated into doughs and subjected to baking tests. The results are given in Table V.

These data show quite definitely that in brews containing yeast extract, the "matured" yeast was superior in baking quality to fresh yeast. There was no apparent improvement in the baking quality of the medium due to brew fermentation; in fact, the brews containing

TABLE V  
EFFECT OF BREW FRACTIONATION ON BAKING QUALITY

SAMPLE <sup>a</sup>	LOAF VOLUME <sup>b</sup>	PROOF HEIGHT	GRAIN AND TEXTURE (MAXIMUM 6)
	cc	mm	
Original brew	685	9	5
Fresh yeast and fermented medium	620	0	3
Fresh yeast and fresh medium	630	0	3
Yeast from brew and fresh medium	700	10.5	5

<sup>a</sup> All brews and all the media contained 1% yeast extract.

<sup>b</sup> Least significant difference 24 cc.

fresh medium gave very slightly larger loaf volumes than the corresponding brews with fermented medium. This difference was, however, not statistically significant.

*Properties of Yeast after Brew Fermentation with and without Yeast Extract.* To indicate changes which might be occurring in yeast during brew fermentation with yeast extract, dry-weight and Kjeldahl nitrogen determinations were made. "Succinate" brews with and without the addition of 1% yeast extract were prepared for analysis in the following manner:

Seventy-five-milliliter aliquots of the brews were centrifuged to separate the yeast cells from the medium. The supernatant was discarded and the cells resuspended in 75 ml. water. The centrifuging and washing procedures were repeated until the washings were free of chloride ion (determined by testing with silver nitrate solution). The cells were washed twice more, then suspended in water and made to a volume of 50 ml. Five milliliters of this suspension were used for a Kjeldahl nitrogen determination and 5 ml. for a dry-weight determination by the method of Dale *et al.* (7).

The mean nitrogen content of the yeast isolated from a brew made without yeast extract was 8.35% (dry-weight basis) compared to 9.58%

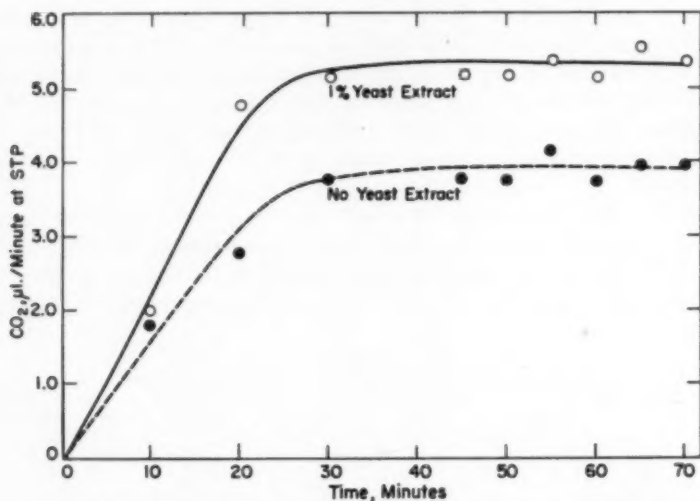


Fig. 1. Influence of yeast extract on the gas production of yeast isolated from fermented brews. Determinations were made in Warburg respirometers at 38°C. with 3.0 ml. of yeast suspension (1.32 mg. yeast, dry-matter basis) in succinate buffer, pH 5.0, containing 16.2 mg. glucose.

for yeast from the brew containing 1% yeast extract. Determinations conducted on duplicate brews showed that the presence of 1% yeast extract in the brew had no effect on yeast dry weight.

Using aliquots of the same washed cell suspensions that were used for dry-weight and nitrogen analyses, gas production rates for the two yeasts were determined in a Warburg constant-volume respirometer. The Warburg flasks contained 1.32 mg. yeast in 1 ml. water, 1.5 ml. of 0.05M succinate buffer, pH 5.0; 0.3 ml. of 0.3M glucose, and 0.2 ml. water in the center well. The air in the flasks was replaced by alternate evacuation and filling with nitrogen. Carbon dioxide production for the two yeasts, determined at 38°C., is given in Fig. 1.

The addition of yeast extract to the brew gave a marked stimulation to the fermentative ability of the yeast. The yeast from the brews made with or without yeast extract gave maximum gas production rates after about 30 minutes. However, the yeast isolated from the brew containing yeast extract gave a maximum which was some 30% higher than the control yeast.

Other properties of the yeast in brews were investigated to determine whether they were influenced by the presence of yeast extract. These included yeast population, yeast cell size, and the population of budding cells. In no case were statistically significant differences found between yeasts in brews with and without added yeast extract. Slow aeration had no consistent and significant effect on these properties.

The addition of aureomycin or penicillin G to brews in concentrations of 100  $\mu$ g/ml did not cause any appreciable change in the bacterial plate count. From this it was concluded that the bacteria were probably in the resting state and thus did not contribute materially to the brews. Neither penicillin G nor aureomycin had any effect on the baking quality of the brews to which they were added.

*Influence of Various Sugars in the Brew.* The rate of utilization of various sugars in the brew was followed by measuring the changes in their concentration and the gas production. In addition, the baking quality of the brews prepared with various added sugars was studied, as well as the relative rates of disappearance of glucose, fructose, and maltose from the dough.

"Succinate" brews were prepared with and without the addition of 1% yeast extract and containing 6.25 g/100 ml sucrose, glucose, and maltose respectively. Two other brews were prepared, one with 1% yeast extract and one without, and both containing 6.25 g/100 ml of a maltose-glucose mixture (9:1).

The gas production and sugar levels obtained from the brews are given in Figs. 2, 3, and 4.

Yeast extract stimulated the production of gas from sucrose (Fig. 2). No sucrose was detected in the brew when the first sample was taken 5 minutes after the ingredients were mixed. Glucose and fructose were

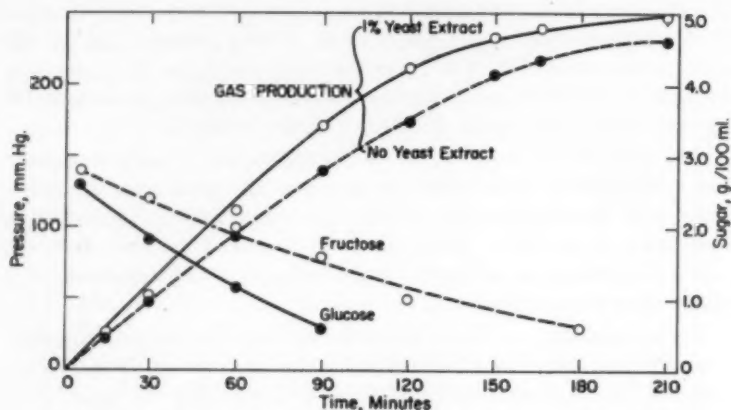


Fig. 2. Gas production and changes in glucose and fructose content of 100 ml. of "succinate" brews (pH 5.5) containing 6.25 g. each of sucrose (equivalent to 5.7% of total brew) and yeast. Sugar levels are given only for the brew containing yeast extract.

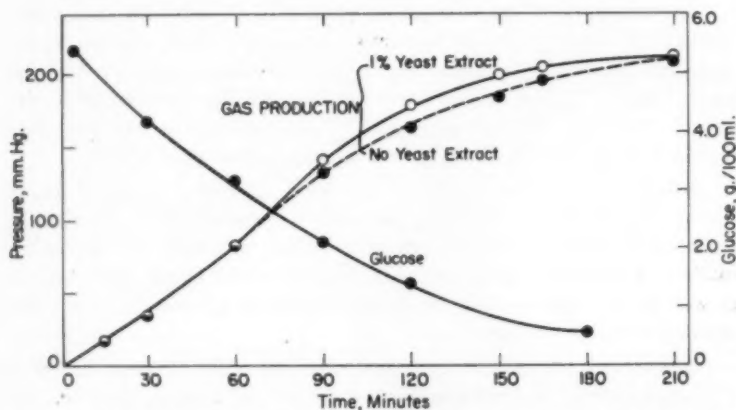


Fig. 3. Gas production and changes in glucose content of 100 ml. of "succinate" brews (pH 5.5) containing 6.25 g. each of anhydrous glucose (equivalent to 5.7% of total brew) and yeast. Glucose concentrations are given only for the brew containing yeast extract.

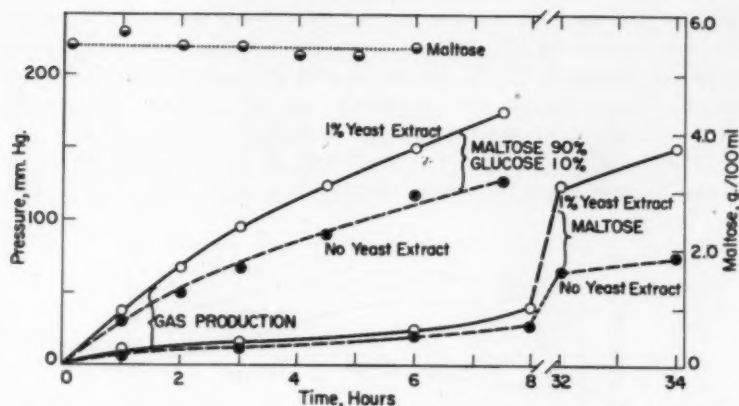


Fig. 4. Influence of yeast extract on gas production by 100 ml. of "succinate" brews made with maltose and with a maltose-glucose mixture (9:1). The brews contained 6.25 g. of yeast and of the respective sugars and were fermented at 37°C. The maltose levels are given for the brew made with maltose and yeast extract.

fermented simultaneously, although glucose was utilized at a greater rate. Gas production was essentially complete after 210 minutes. Yeast extract did not increase the rate of gas production from brews containing glucose (Fig. 3) as much as it did in the case of sucrose. When maltose alone or maltose with yeast extract was used in brews, very little gas was produced for the first 8 hours, and the sugar utilization was so small as to be unmeasurable with the analytical techniques used (Fig. 4). After 32 hours' fermentation, stimulation of maltose fermentation by yeast extract was observed. Even after 34 hours' fermentation, gas production did not appear to be complete, as evidenced by the slope of the lines. This shows that maltose is poorly fermented by baker's yeast which has been grown on a sucrose medium. The enhancement of the fermentation of maltose by glucose which has been previously observed (19) is clearly evident in Fig. 4. The pressures developed after 8 hours are far too great to be due exclusively or even largely to the gas production from glucose. Yeast extract stimulated the gas production from the maltose-glucose mixture.

*Influence of the Sugar Used in Brew Preparation on Changes in Concentration of Individual Sugars in the Dough.* The sugar used in brew preparation could conceivably influence the relative rates of fermentation of glucose, fructose, and maltose in the dough.

"Succinate" brews containing 1% yeast extract and 6.25 g/100 ml of glucose, sucrose, maltose, and a maltose-glucose mixture (9:1) respectively, were prepared in the usual way except that the brews con-

taining maltose and maltose-glucose were fermented for 12 hours. Doughs containing 5% sucrose (flour basis) made from these brews were sampled at 4, 15, 30, 45, 60, 75, and 90 minutes, and chromatographic sugar analyses were performed on each sample. The results of these sugar determinations are given in Figs. 5, 6, 7, and 8.

The doughs from the brews made with sucrose and glucose (Figs. 5 and 6) gave sugar utilization curves which were very similar. The

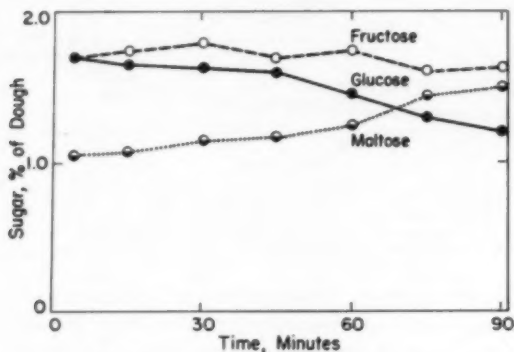


Fig. 5. Changes in the glucose, fructose, and maltose contents of doughs fermented at 30°C. which were prepared from brews made with sucrose (6.25 g/100 ml). Sugar levels are based on dough weight corrected to 40% moisture. The doughs initially contained 3% yeast and 5% sucrose on a flour basis. The sucrose added was equivalent to 1.78% of glucose and 1.78% fructose on a dough basis.

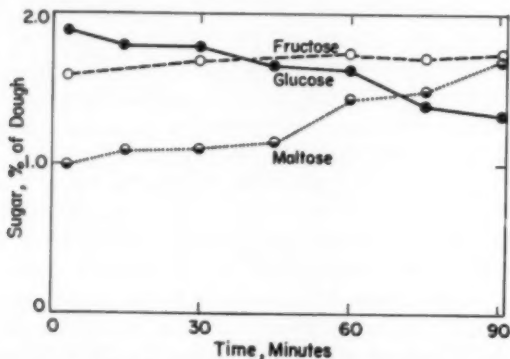


Fig. 6. Changes in the glucose, fructose, and maltose contents of doughs fermented at 30°C. which were prepared from brews made with anhydrous glucose (6.25 g/100 ml). Sugar levels are based on dough weight corrected to 40% moisture. The doughs initially contained 3% yeast and 5% sucrose on a flour basis. The sucrose added was equivalent to 1.78% of glucose and 1.78% fructose on a dough basis.

glucose concentration decreased appreciably, whereas the fructose level decreased only very slightly. The maltose level in these two doughs increased from approximately 1.0 to 1.5% at a fairly uniform rate. Glucose decreased at a slightly faster rate in the dough made from the "maltose-glucose" brew (Fig. 8) than in the one made from the "maltose" brew (Fig. 7). In contrast to the situation in doughs made from sponges where the maltose concentration decreased continuously, the

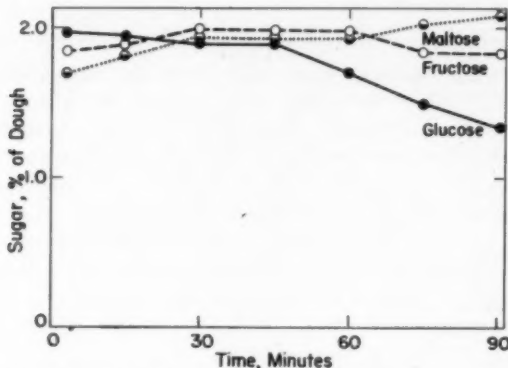


Fig. 7. Changes in the glucose, fructose, and maltose contents of doughs fermented at 30°C. which were prepared from brews made with anhydrous maltose (6.25 g/100 ml). Sugar levels are based on dough weight corrected to 40% moisture. The doughs initially contained 3% yeast and 5% sucrose on a flour basis. The sucrose added was equivalent to 1.78% of glucose and 1.78% fructose on a dough basis.

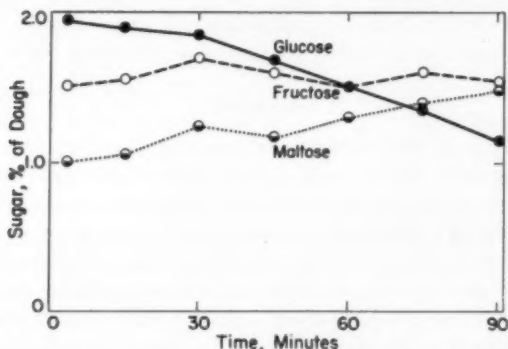


Fig. 8. Changes in the glucose, fructose, and maltose contents of doughs fermented at 30°C. which were prepared from brews made with a mixture of maltose and glucose (9:1; 6.25 g/100 ml). Sugar levels are based on dough weight corrected to 40% moisture. The doughs initially contained 3% yeast and 5% sucrose on a flour basis. The sucrose added was equivalent to 1.78% of glucose and 1.78% fructose on a dough basis.

maltose level in doughs made from brews increased during the 90-minute fermentation period.

*Influence of Different Sugars Added to Brews on Baking Quality and on Sugar Content of the Bread.* All the brews used for the sugar determinations just described were subjected to baking tests and the resulting bread crumb analyzed for its content of glucose, fructose, and maltose. The results of these baking tests and sugar determinations are given in Table VI.

TABLE VI  
INFLUENCE OF BREW SUGAR ON BAKING QUALITY AND SUGAR CONTENT OF BREAD

SUGAR IN BREW <sup>a</sup>	LOAF VOLUME <sup>b</sup>	PROOF HEIGHT	GRAIN AND TEXTURE (MAXIMUM 6)	GLUCOSE <sup>c</sup>	FRUCTOSE <sup>c</sup>	MALTOSE <sup>c</sup>
	cc	mm		%	%	%
Sucrose	685	8	5	1.0	1.4	1.4
Glucose	675	8	5	1.1	1.6	1.7
Maltose	615	—2	3	1.2	1.8	2.2
9:1 Maltose-glucose	680	7	5	1.0	1.5	1.5

<sup>a</sup> 6.25 g/100 ml.

<sup>b</sup> Least significant difference 24 cc.

<sup>c</sup> Results based upon 40% bread crumb moisture.

Sucrose, glucose, and the maltose-glucose mixture were equally satisfactory in the brews. However, maltose alone caused a marked drop in baking quality. The concentration of maltose in bread made from all brews was about three times higher than that normally found in bread made by the sponge and dough procedure (11). The glucose and fructose levels, however, approximated those found in sponge and dough bread made with 5% added sucrose (flour basis) (11).

### Discussion

The studies on the effect of various sources of yeast nutrients on the baking quality of succinate brews revealed that yeast extract was superior to an aqueous extract of flour, hydrolyzed casein, and various synthetic nutrients. Since the succinate brew did not contain a source of nitrogen, at least part of the stimulatory effect of yeast extract was undoubtedly due to its nitrogenous constituents. Brew fractionation studies showed that the improving action of yeast extract was reflected in an enhanced fermentative power in the yeast. The yeast had a higher nitrogen content than that prepared without yeast extract, but there was no increase in yeast population in the dry weight of cells or in the volume of the packed cells. The active constituents were confined to the dialyzable portion of the extract but most of the efforts

to fractionate the extract resulted in the removal of part, but not all, of these substances.

Brews made with maltose gave poor baking results because of their very limited ability to ferment maltose produced in the doughs through amylase action. Very little gas was produced by the doughs and they yielded loaves of lower volume and higher maltose content than those obtained with brews made with sucrose and glucose, or with a maltose-glucose mixture (9:1). The maltose levels in the breads made by the brew process are much higher than those obtained by Koch *et al.* (11) with bread made by the sponge and dough process; in fact, the maltose levels of the bread made with brews corresponded very closely with those found by these workers for bread made by the straight dough method.

These results are in accord with information in the literature. It is well known that in solutions of pure maltose, baker's yeast has a long induction period. Schultz and Atkin (19) found that the addition of a relatively small proportion of glucose sharply reduces the induction period in the fermentation of maltose; a small quantity of sucrose, which is rapidly hydrolyzed to glucose and fructose, had a similar effect. In the present studies, the addition of a small quantity of glucose along with maltose in making the brew resulted in a marked increase in gas production of the doughs, an increased loaf volume, and a lower concentration of maltose in the bread.

The presence of maltose and small amounts of glucose are apparently essential to induce maltose fermentation. In brews made with glucose, sucrose, or maltose, these requirements are not met, so that doughs made with brews ferment maltose very poorly. In the sponge and dough method of breadmaking, reducing sugars and maltose are both present in the sponge because it contains flour. Although glucose and fructose are fermented preferentially to maltose, the yeast becomes adapted to the fermentation of maltose when these sugars reach a low concentration. Koch *et al.* (11) found that in a dough made from a sponge, the yeast can ferment maltose rapidly in the presence of relatively high concentrations of glucose or fructose. In contrast, maltose is not fermented in a straight dough until the supply of glucose and fructose is reduced to a low level.

#### Acknowledgment

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# THE DISTRIBUTION OF ASH IN THE WHEAT KERNEL<sup>1</sup>

J. J. C. HINTON

## ABSTRACT

The range in the proportion of the total ash found in various hand-dissected parts from four wheats (Thatcher, Vilmorin 27, an Argentinian variety, and an Egyptian variety) was: aleurone layer, 56.4–60.2%; endosperm, 20.3–25.9%; pericarp, testa, and hyaline layer, 7.3–9.8%; scutellum, 5.5–8.2%; embryo, 2.8–4.0%.

The gradient in ash content, which diminished from outer to inner layers of the endosperm, was not identical in the four wheats leading to differences in the curves plotting mean ash content of the endosperm against "extraction rate," the mean ash content rising more rapidly in two of the wheats than in the other two.

The ash content of the central endosperm (about 50% of the total endosperm) of 15 commercial wheat samples had no apparent relationship to the ash content of the whole kernel or to kernel weight.

Comparison with other published results shows good agreement between four investigations based on hand-separated dry material.

Reliable information on the ash content of the anatomical parts of cereal kernels can only be obtained on material separated in the dry state, for it has been shown, for example, that bran cleaned from endosperm by maceration methods has lost about 80% of its ash (5). The ash content of hand-separated bran, germ, and regions of the endosperm of wheat has been determined by Morris, Alexander, and Pascoe (15,16) and of hand-cleaned bran, germ, and total endosperm of wheat by Mambish (14). Similar figures for hand-separated fractions of the maize kernel were obtained by Hopkins, Smith, and East (8) and by Earle, Curtis, and Hubbard (2) and on hand-separated fractions of the sorghum kernel by Hubbard, Hall, and Earle (9).

This paper reports the ash content of hand-dissected fractions, namely pericarp and testa, aleurone layer, regions of the endosperm, embryo, and scutellum of four types of wheat from which its distribution within the kernel has been calculated, together with the ash content of the central endosperm of a range of wheat samples.

## Materials and Methods

Four wheats, the first three of which represent classes of interest in English conditions, were examined in detail:

Thatcher: grown in Canada, hard, red, vitreous endosperm.

Vilmorin 27: grown in England, soft, red, mealy endosperm.

<sup>1</sup> Manuscript received May 26, 1958. Contribution from The Research Association of British Flour Millers, Cereals Research Station, St. Albans, England.

Argentinian: variety unknown, soft, red, mixed endosperm.

Egyptian: variety unknown, grown at Giza, hard, red, mixed endosperm.

Fifteen wheat samples, identified in Table II, were examined by dissecting the central endosperm only and determining its ash and that of the whole kernel. Most of these were obtained from commercial sources and the varieties were unknown. All were sound and carried no dust in the beard or crease.

The dissected parts are indicated in Fig. 1 and are defined thus: Pericarp and testa: the pericarp, testa, and hyaline layer.

Aleurone layer: free from endosperm.

Endosperm 1: adjoining the aleurone layer, 70-100  $\mu$  in depth.

Endosperm 2, 3, etc.: succeeding regions towards and concentric with the crease.

Endosperm 5 (Argentinian and Egyptian), 6 (Thatcher), 7 (Vilmorin 27): adjoining the aleurone layer in the crease.

Central endosperm: 40-50% of the endosperm taken from the central part of the cheek and back regions.

Embryo: the embryonic axis.

Scutellum.

The kernels, held in a small "pin vise," were dissected in the natural untreated state by straightforward methods using a dissecting microscope and small dissecting needles and knives of various shapes and sizes. The pericarp and testa portion was first removed after the moisture content was raised by placing the kernels in a moist air chamber for two or three days; then these layers could be stripped cleanly away. The kernel was allowed to dry at ordinary temperatures for several hours, and the removal of the aleurone layer was facilitated by blowing a gentle stream of moist air onto it. Any endosperm adhering to the aleurone layer was removed by light scraping. The endosperm fractions were then cut away in turn, proceeding towards the crease. The central endosperm (Table II) was drilled from transversely cut kernels using a triangular-headed, mounted needle which was rotated between the fingers.

The dissected material was allowed to reach equilibrium moisture content with the air for two or three days and was weighed as air-dry material. Forty to fifty kernels were dissected for each determination. The end portions, which were difficult to handle, yielded fractions of unsatisfactory purity and were not included in the material for ash estimations. To determine the proportion of the anatomical parts, ten half-kernels, cut longitudinally, were dissected completely.

Duplicate samples, repeated where necessary, were ashed on a micro

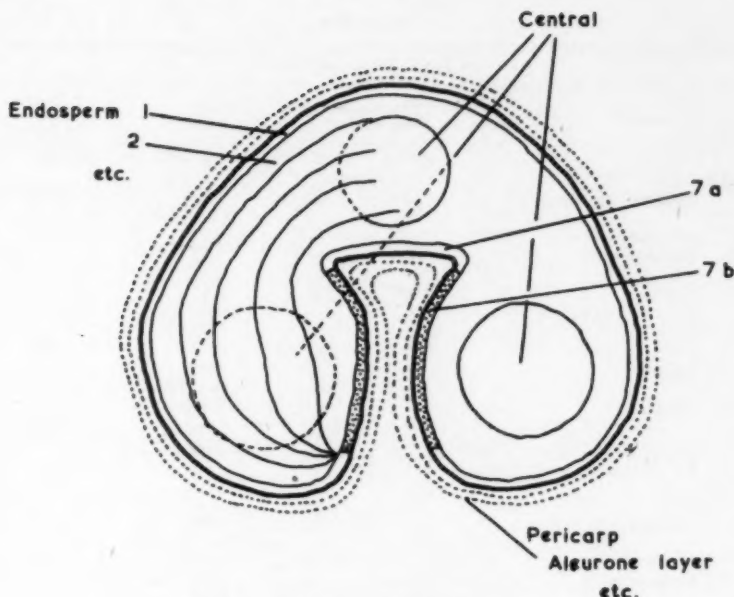


Fig. 1. Dissected regions of the endosperm.

scale at 600°C. for 16 hours using silica boats in a small tube furnace. The ash weighed varied from 0.1 to 5 mg., and reasonable agreement was obtained with the standard method in use in these laboratories. With the smaller amounts of ash coming from the endosperm fractions, a rather high error of  $\pm 7\%$  was experienced. For the most part, this could be accounted for by the errors of weighing and manipulation.

All the wheat samples appeared to be free from dust in crease and beard; however, a small number of those of high ash content (shown in Table II) were washed five times with water and dried, and their ash determinations were repeated. There was no significant effect on the figures.

Moisture was determined by heating coarsely ground whole wheat for 4 hours and other material for 2 hours at 120°C. The moisture content of air-dried material has been found to be fairly steady under conditions in our laboratory, and it was determined on bulked samples taken from the various fractions rather than on each sample ashed.

### Results

The anatomical composition, ash content of dissected fractions, and

TABLE I  
THE ASH CONTENT OF DISSECTED FRACTIONS AND ITS ANATOMICAL DISTRIBUTION  
IN FOUR TYPES OF WHEAT

FRACTION	PROPORTION OF THE KERNEL		ASH CONTENT <sup>a</sup>	PROPORTION OF TOTAL ASH OF KERNEL
	%	%		%
Thatcher				
Pericarp and testa		8.2	1.70	7.3
Aleurone layer		6.7	17.22	60.2
Endosperm 1	3.1		1.34	
2	7.5		0.52	
3	11.0		0.43	
4	11.8		0.39	
5	21.2		0.39	
6	26.9	81.5	0.49	20.3
Embryo		1.6	4.83	4.0
Scutellum		2.0	7.83	8.2
		100.0		100.0
Entire kernel direct determination			1.68	
calculated from fractions			1.91	
Vilmorin 27				
Pericarp and testa		8.0	1.96	8.6
Aleurone layer		7.0	14.72	56.4
Endosperm 1	11.0		1.01	
2	10.4		0.71	
3	14.9		0.44	
4	17.9		0.44	
5	11.9		0.37	
6	10.0		0.42	
7a	2.6		0.77	
7b	3.8	82.5	1.00	25.9
Embryo		1.0	5.36	2.9
Scutellum		1.5	7.59	6.2
		100.0		100.0
Entire kernel direct determination			1.63	
calculated from fractions			1.83	
Argentinian				
Pericarp and testa		9.5	1.91	9.8
Aleurone layer		6.4	17.03	58.7
Endosperm 1	4.5		1.87	
2	4.4		0.59	
3	26.6		0.45	
4	24.6		0.38	
5	21.3	81.4	0.40	21.9
Embryo		1.3	4.83	3.4
Scutellum		1.4	8.20	6.2
		100.0		100.0
Entire kernel direct determination			1.89	
calculated from fractions			1.86	

(Continued)

TABLE I (Cont.)

FRACTION	PROPORTION OF THE KERNEL		ASH CONTENT <sup>a</sup>	PROPORTION OF TOTAL ASH OF KERNEL
	%	%		%
Egyptian				
Pericarp and testa		7.4	2.07	7.93 <sup>b</sup>
Aleurone layer		6.7	14.37	
Endosperm 1	3.4		1.19	59.9
2	8.0		0.64	
3	24.0		0.26	
4	25.1		0.33	
5	23.6	84.1	0.52	22.3
Embryo		1.3	3.51	2.8
Scutellum		1.5	5.87	5.5
		100.0		100.0
Entire kernel direct determination			1.52	
calculated from fractions			1.61	

<sup>a</sup> 14% moisture basis. The approximate air-dry figures for the fractions were: pericarp and testa 10.7%; aleurone layer 10.7%; embryo 8.7%; scutellum 9.3%; endosperm 13%; whole kernel 13%.

<sup>b</sup> Weighted mean.

TABLE II  
ASH CONTENT OF KERNEL AND CENTRAL ENDOSPERM AND AVERAGE  
KERNEL WEIGHT OF VARIOUS WHEAT SAMPLES

ORIGIN OF SAMPLE	ASH OF KERNEL <sup>a</sup>	ASH OF CENTRAL ENDOSPERM <sup>a</sup>	AVERAGE KERNEL WEIGHT
	%	%	mg
1. Iraq	1.38	0.40	37
2. Central India (var. Pissi)	1.44	0.36	48
3. Argentina, Bahia Blanco	1.51	0.32	37
4. Egypt	1.52	0.30	58
5. England (var. Holdfast)	1.54	0.38	47
6. Uruguay	1.54	0.29	45
7. Pakistan, Punjab	1.56	0.27	49
8. England (var. Bersee)	1.57	0.38	52
9. Pakistan, Karachi	1.58	0.46	46
10. Canada (var. Durum)	1.61	0.34	60
11. England (var. Vilmorin 27)	1.63	0.42	69
12. India, Pusa	1.64	0.26	60
13. Egypt, Giza	1.64	0.40	58
14. England (var. Atle)	1.67	0.36	48
15. Canada (var. Thatcher)	1.68	0.39	36
16. Hungary	1.71	0.27	49
17. Argentina	1.89	0.40	36
18. Argentina, Rosafe	1.90	0.28	38
19. Iraq	1.91	0.47	42

<sup>a</sup> 14% moisture basis.

distribution of ash within the kernel for the four wheats dissected in detail are presented in Table I.

In Table II are given the ash content of the wheat and central endosperm and the average kernel weight of 19 wheats, including

those of Table I. The figures shown for samples 2, 5, 9, 16, 18, are the averages of six determinations.

Figure 2 expresses, in the form of curves, the relationship between the "extraction rate" and the mean ash content of the dissected endosperm of the four wheats of Table I and compares this with the same

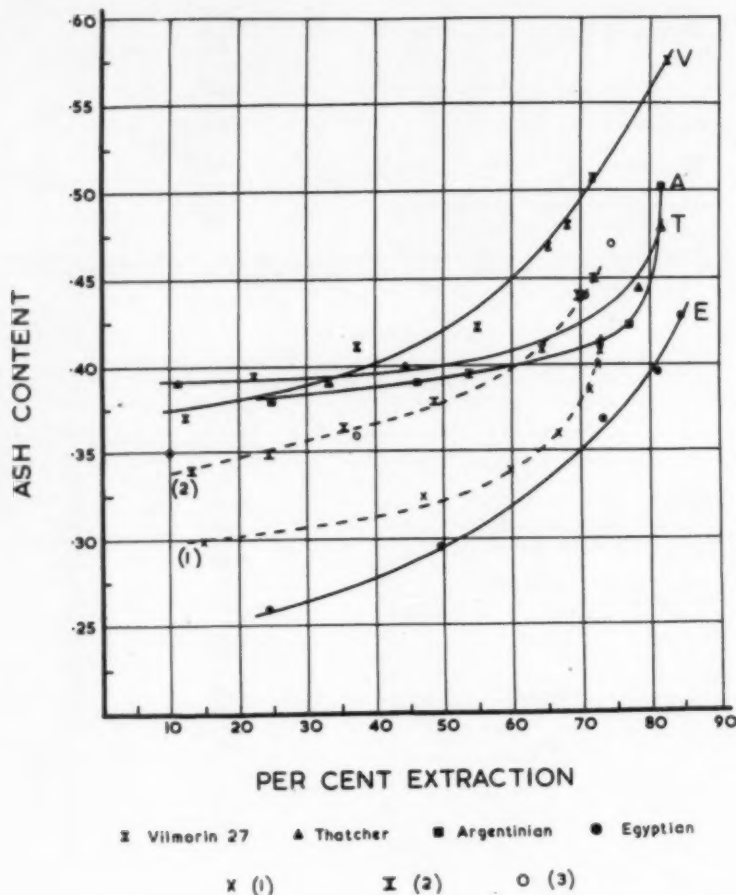


Fig. 2. Relationship between mean ash content (%) and percent extraction for dissected endosperm and milled flour 1, Kent-Jones and Amos (12); Table XCVII, p. 173. English commercial milling of mixed grist comprising seven wheats. 2. Research Association of British Flour Millers. Unpublished report. Laboratory milling of No. 1 Manitoba Northern wheat. 3. Jackson, Doherty, and Malone (10); Table I, p. 553. Canadian commercial milling of hard Western Canadian wheat.

relationship for three milled flours. The steps on the extraction rate coordinate were fixed by the successive additions of endosperm fractions, in order of increasing ash content, irrespective of anatomical relationships; the same principle was followed for the milled flours.

### Discussion

The four wheats examined in detail gave results which agree well with each other. The sum of the ash content of the dissected fractions differs from the figure obtained for the whole kernel by from 2 to 12% in the four samples. This represents the errors of sampling, dissection, and ash estimation.

The greater part of the ash of the kernel, 56.4% in Vilmorin 27 to 60% in Thatcher, is contained in the aleurone layer. The endosperm contains from 20.4% in Thatcher to 25.9% in Vilmorin 27. The complete embryo accounts for 8.3–12.2%, with the concentration of ash 40–70% greater in the scutellum than in the embryo fraction. The fibrous pericarp and testa contain less than 10% of the ash of the kernel.

The endosperm fractions in the four samples are not completely comparable; the dissections were not quite the same, and the fractions represent slightly different divisions of the endosperm. The Argentinian and Egyptian, however, are sufficiently close to be compared, and the figures suggest that the lowest ash content is found in a slightly different region in these two wheats, region 4 in the Argentinian and 3 in the Egyptian. The differences in ash content are small but are outside the error experienced. No ready explanation can be seen; the Argentinian was much leaner than the Egyptian, the average weight of the best kernels being 36 mg. compared with 58 mg. Any direct connection here seems improbable since in Vilmorin 27, with a kernel of 69 mg., the ash gradient in the endosperm appears to be more like the leaner Argentinian than the Egyptian.

Incidentally, the Egyptian sample was chosen because it had produced a 70% extraction flour with an unusually high ash of 0.78% in a laboratory test milling. The ash content of the endosperm, however, is low; so the high figure for the flour must be the result of the milling characteristics of the wheat, possibly produced by the inclusion of a proportion of aleurone layer in the flour.

Figure 2 suggests further differences among the endosperms of the four wheats. The curves for Thatcher and the Argentinian are similar in shape and show that the mean ash content increases little up to an extraction of about 77%. Thereafter it rises much more steeply as the outer endosperm is included. The Argentinian differs from Thatcher,

however, in that the rise above 77% is greater, which causes a markedly greater rise in mean ash content over the whole extraction range. Both Vilmorin 27 and the Egyptian differ from these in that the rise in mean ash content is more rapid in the lower extraction ranges, which results in a more uniform over-all rise without the steepening above 77%. The method of constructing the curves takes no account of the anatomical relationship of the fractions of the endosperm, and when Vilmorin 27 and the Argentinian are examined in this way, they are seen to be unlike, although, as mentioned above, the lowest ash content may be in similar regions of the endosperm in the two wheats.

Curve 2 is based on the laboratory milling of No. 1 Manitoba Northern and is also an approximate fit for data obtained from a commercial milling of hard Western Canadian wheat. It is, therefore, probably directly comparable with the curve for Thatcher endosperm, and its steeper slope indicates the effect of the inclusion of bran fragments. Curve 1 represents a typical English flour from a mixed grist comprising seven types of wheat. The similarity shown by these curves with those for dissected endosperm must be, in part, fortuitous, since the upward turn which occurs now at a lower extraction of about 67% follows, in this case, from the increasing inclusion of bran and germ fragments; moreover, in the patent range, some degree of removal of cell-wall material occurs. Nevertheless, the endosperm curve must underlie the flour curve, and such wheats as Vilmorin 27 and the Egyptian, in which the mean ash content of the endosperm rises appreciably in the lower ranges, must place a more exacting limit on what can be achieved in practice than those such as Thatcher and the Argentinian.

The endosperm of Vilmorin 27 was dissected in somewhat greater detail than that of the remaining wheats. Endosperm 7 in Table I adjoined the aleurone layer in the region of the crease, 7a at the central inner part, and 7b at the sides (Fig. 1). This subdivision was made because of the modified nature of the aleurone layer in the central part corresponding to 7a (1). Gordon (3) has drawn attention to the probability that the aleurone layer remains meristematic, forming new endosperm cells, until a relatively late stage in the development of the endosperm. Observations which support this suggestion have been published by Sandstedt (17), and similar observations have been made by the author.

The author's own observations also suggest that the difference between the modified aleurone cells near the vascular strand and the normal aleurone cells occurring elsewhere appears at an early stage in kernel development. Ten days after flowering, the normal cells were long and narrow; the shorter axis was the radial one and had the typi-

cal appearance of actively dividing cells. There was evidence in the outer two or three rows of endosperm cells of a radial alignment with the aleurone cells; these endosperm cells were only slightly differentiated, with traces of starch. At this time the modified aleurone cells were more nearly square in shape, and there was no obvious radial alignment in the associated endosperm cells, which were further developed with well-formed starch grains. It is probable, therefore, that the endosperm described as 7a was much more mature than 7b and may have had a different history, possibly as part of the original endosperm division. The difference in ash content does reflect this difference in character, though it might well have been expected to be larger. It is probable that the dissection was only imperfect at this point since no clear demarcation of the endosperm exists, and that would tend to obscure any difference.

It seems quite probable, therefore, that the gradient in ash content, and in many other constituents throughout the endosperm, is linked with the difference in stage of development of the cells, increased concentration of ash and decreased concentration of starch being characteristics of the less mature cells occurring in the regions lying close to the normal aleurone layer. It is of interest to note that for reasonably comparable dissections of Vilmorin 27, the concentration of ash, protein, thiamine, and niacin in the outer endosperm is, respectively, 2.12, 2.3 (7), 3.0 (7), and 3.2 (6) times that in the inner endosperm.

Table II contains the ash content of the central endosperm of a number of wheats, including those of Table I, with a total ash ranging from 1.38 to 1.91%. There appears to be no relationship between the ash of the kernel and that of the central endosperm. Sample 1 with a central endosperm containing 0.40% ash had a total ash of 1.38%, whereas in sample 18 the endosperm was 0.28%, and the total ash was 1.90%. The average weight of the dissected kernels is included in the table as an index to the degree to which the endosperm has been filled because this would influence the proportion of the total endosperm removed as "central." The extent of the filling of the endosperm might also affect its ash content. However, it does not appear that kernel size influences the figures obtained; sample 18 had a very lean kernel, a high ash, but low endosperm ash, whereas sample 9, with a medium kernel weight and ash content, had a high endosperm ash. A full examination of the figures failed to reveal any correlation between the ash of the kernel, ash of the central endosperm, and kernel weight; the coefficient, for example, between ash of the kernel and central endosperm was  $r = +0.12$ .

*Comparison with Other Published Results.* Shetlar *et al.* (19) gave

figures for the ash content of the bran layers of wheat separated in detail by maceration methods. When recombined to compare with the pericarp and testa fraction of Table I and expressed on a 14% moisture basis, the average ash content is 1.33% compared with an average of 1.91% for the dry dissected material from Table I. The difference may be due to loss of soluble minerals during the maceration. This may also account for the larger difference in ash of the aleurone layer as determined by the two methods; the maceration method gave an average figure of 6.2% compared with an average of 15.8% from Table I.

Mambish (14), in an extensive study of some 150 U.S.S.R. wheats, obtained figures for combined pericarp, testa and aleurone layer, total germ, and endosperm; these were separated by a combination of milling and dissection methods. Morris *et al.* (15, 16) and Kazakov (11) also have given figures relating to total germ and endosperm. A comparison of these three sets of figures, expressed on a 14% moisture basis, with those obtained in the present study is given in Table III, from which it is apparent that agreement between the figures obtained by dry separation methods is good. In addition, several investigations have been based on indirect methods of dry separation and calculation. Typical figures obtained by Grischenko (4) in this way are included for comparison in Table III.

Morris *et al.* (16) made a study of regions of the endosperm by a slightly different dissection technique, to which the results described here are largely complementary. Their figure for the central endosperm of Thatcher was 0.38% compared with 0.39% from Table I. In the central endosperm of the soft wheat Trumbull they found 0.24%, which compares with the lowest figure of Table II, 0.26% for the Indian wheat (sample 12).

The conclusion drawn from the results of Table II is that there is no relationship between the ash of the wheat and that of the central endosperm. That seems to be at variance with the experience of Sherwood and Bailey (18), who, in an examination of 148 wheat samples, found a strong positive correlation between the ash of the wheat and that of the straight-run flour milled therefrom. The possibility that this may follow from the inclusion of parts of the kernel other than endosperm is remote since straight-run flour can be produced with ash slightly lower than that of total dissected endosperm (16). The anomaly can be explained if the form and extent of the gradient in the endosperm varies from one wheat to another, so that the ash of the central endosperm as dissected bears no close relation to the ash of

TABLE III  
COMPARISON OF RESULTS OBTAINED BY DIFFERENT AUTHORS

	HINTON	MAMBISH	KAZAKOV	MORRIS, ALEXANDER, AND PASCHE	GRIICHENKO
	%	%	%	%	%
Combined pericarp, testa, and aleurone layer	14.1-15.9 7.92-8.6	13.7-20.0 5.29-9.48			14.4-14.6 7.15-8.70
Proportion of kernel Ash content					
Total germ	2.5-3.6 4.79-6.72	2.0-3.1 4.27-6.22	3.4-3.8 4.48-6.03	3.2-3.4 5.35-5.87	2.5-3.2 3.66-4.35
Proportion of kernel Ash content					
Total endosperm	81.4-84.1 0.43-0.50	77.0-84.1 0.22-0.46	75.9-79.2 0.36-0.48	78.7-80.3 0.36-0.42	80.7-83.1 0.23-0.31

the endosperm as a whole or to that of the wheat. The suggestion of such a difference was seen in the figures for the Argentinian and Egyptian wheats in Table I, and the idea is supported by the finding of Sherwood and Bailey that the ash content of the wheat is more weakly correlated with that of a mill stream carrying low ash endosperm than it is with the straight-run flour. At the same time, however, the curves of Fig. 2, notably those for the Argentinian and Vil-morin 27 wheats, show that the ash content of the endosperm as a whole may not be related to that of the kernel, a conclusion which can be drawn also from other published results for Tenmarq and Thatcher wheats (16).

The pattern of distribution of ash found here for wheat with about 60% in the aleurone layer is not followed in all cereals. In maize about 80% of the total is found in the germ (2.8), and since the scutellum accounts for 90% of the germ (6), most of it must be in the scutellum. This, however, follows mainly from the larger size of the scutellum in maize, average 12% of the kernel (6) compared with 1.6% in wheat; the ash of the germ in maize, average 10% (2.8) is of the same order as that of the scutellum of wheat, average 8.5%, as found here. There is evidence of differences in ash content of different regions of the endosperm and of a relatively high ash in the aleurone layer of maize (8), but the figures suggest that the latter must be at least 50% lower in value than it is in wheat. It seems clear, however, that the underlying composition of the different parts is similar in the two cereals and that the different distribution pattern follows essentially from the difference in anatomical composition.

Ash distribution in the kernel of sorghum, about 70% in the germ, is very similar to that in maize. The structure and anatomical composition of the two cereals is much alike; the germ in sorghum amounts to 10% of the kernel compared with an average figure of 12% in maize (9).

Figures for other cereals are not available, but there are indications that in rice the ash distribution is similar to that in wheat since about 78% of it is lost in polishing (13), and a strong correlation has been reported between thickness of the aleurone layer and ash content of the kernel (20).

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## THE ESTIMATION AND LOCATION OF METHOXYHYDROQUINONE GLYCOSIDES IN THE WHEAT GRAIN<sup>1</sup>

D. G. H. DANIELS

### ABSTRACT

The methoxyhydroquinone (MHQ) glycosides were determined in wheat and milling fractions with the use of the 2:6-dichloroquinone-4-chloroimide reagent. The germ, which contained 0.35-0.47% MHQ, accounted for 60-70% of the total in the grain, and the bran, which contained 0.02-0.04%, accounted for a further 20%. The presence of MHQ monoglucoside was not confirmed. The parallelism between MHQ and thiamine contents of fractions suggested that these substances have a similar distribution in the wheat grain.

In recent years, methoxybenzoquinone has been recognized among the products of the fermentation of wheat germ by yeast (3,4,12), and a precursor, methoxyhydroquinone glucoside (MHQ glucoside), has been isolated from unfermented germ (1). It has been stated that the MHQ derivative occurs only in the germ (2).

It was hoped that a method of determining this substance in flour and other wheat products would allow estimates to be made of the amount of germ contained in them. For this purpose, the reaction of MHQ glucoside with 2:6-dichloroquinone-4-chloroimide (DCQ) (1) seemed to be most suitable. This reagent has been used for the colorimetric estimation of several phenolic substances. Ettinger and Ruchhoft (5) modified Gibbs' original method so as to improve reproducibility in the estimation of traces of phenols in water supplies, and Scudi (10), Hochberg *et al.* (9), and others have worked out methods for determining pyridoxine in foodstuffs. In these methods, blanks are prepared in the presence of borate, which is an inhibitor of the pyridoxine reaction. Then, at lower pH values than the optimum for phenols, a blue color is formed if pyridoxine is present.

The present method for determining MHQ derivatives in wheat has made use of a borate buffer to eliminate interference by pyridoxine. Variability in results was minimized by control of the time and pH of extraction, the quantity of DCQ employed, and the time and temperature of reaction. Flour samples, even if germ-free, had appreciable MHQ values, and it became evident that bran and perhaps even endosperm contained DCQ-reacting substances. Although the method

<sup>1</sup> Manuscript received February 25, 1958. Contribution from the Research Association of British Flour-Millers, St. Albans, England.

cannot therefore be used, as hoped, to determine the germ content of flour, it can be applied to fractions derived from both normal and modified wheat grains to establish the distribution of MHQ in the grain.

### Determination of MHQ Glycosides

A standard solution of methoxyhydroquinone- $\beta$ -glucoside containing 72  $\mu\text{g}/\text{ml}$  (corresponding to 32.4  $\mu\text{g}$  MHQ/ml) was stable for up to 2 months in the refrigerator. The crystalline glucoside, m.p. 199°–201°C. (decomp.), was prepared by the method of de Jong *et al.* (1) and appeared to be a hemihydrate (found: carbon, 50.1%; hydrogen, 6.3%; glucose, 57.0, 58.3%;  $\text{C}_7\text{H}_7\text{O}_2 \cdot \text{C}_6\text{H}_{11}\text{O}_6$  requires carbon 51.6%; hydrogen, 6.0%; glucose, 59.6%;  $\text{C}_7\text{H}_7\text{O}_2 \cdot \text{C}_6\text{H}_{11}\text{O}_6 \cdot 1/2 \text{H}_2\text{O}$  requires carbon, 50.2%; hydrogen, 6.2%; glucose, 57.9%).

Wheat extracts or MHQ glucoside standards (0–65  $\mu\text{g}$ . MHQ) were diluted to 5 ml. with water or buffer solution, as necessary, so that each tube contained 2 ml. of buffer, pH 9.4. The modified borate buffer solution, proposed by Fearon (6), was employed. The extracts were treated with DCQ solution (0.2 ml., 0.25% w/v in ethanol) and, after being allowed to stand for about 16 hours, preferably in the dark at a constant temperature of 20°C., the color was extracted into 1-butanol (12 ml.). After this had settled for 30 minutes, the butanol was pipetted off, centrifuged, and stabilized against fading by carbon dioxide uptake, by the addition of 0.01N sodium hydroxide in ethanol (0.5 ml.). Absorbances were measured in a Spekker absorptiometer by the use of 2-cm. cells and Ilford filters No. 607 (transmission maximum at 600  $\text{m}\mu$ ). Pyridoxine and thiamine did not give a blue coloration with DCQ under these conditions.

There was a linear relationship between MHQ content and absorbance with MHQ glucoside solutions. Blank values and slopes varied slightly from day to day, but control of the temperature reduced these variations greatly. Recovery factors of 88–98% were observed on different occasions, but no corrections for recovery of added glucoside were made. When measurements on a number of wheat products were examined, the standard error of measurement of MHQ was found to increase steadily with level of MHQ over the range of 5–50  $\mu\text{g}$ . per tube. The standard error of log (MHQ value) was 0.0176, with 42 degrees of freedom, from which it was calculated that the 95% confidence limits for a result based on a pair of readings were approximately  $\pm 6\%$ .

It was convenient to express results in terms of MHQ rather than MHQ glucoside since chromatography has not confirmed the presence

of the latter and has shown more than one DCQ-reacting substance in germ extracts. Table I summarizes the chromatographic experiments. MHQ monoglucoside was not detected in any of the extracts examined, but the similarity of the color reactions of the compound of  $R_F$  0.05 (solvent A) suggests that this is closely related to MHQ glucoside, and

TABLE I  
PAPER CHROMATOGRAPHY OF MHQ GLUCOSIDE AND RELATED COMPOUNDS

SUBSTANCE	R <sub>F</sub>		SPOT COLORS	
	System A	System B	Reagent I	Reagent II
MHQ glucoside	0.38	0.55	Pink	Blue
Arbutin (hydroquinone- $\beta$ -monoglucoside)	0.44		Brown	Blue-green
MHQ	0.90	0.95	Brown	Yellow
Aqueous germ extract	0.05 (strong)	0.06 (strong)	Pink	Blue
	0.14 (weak)	0.18 (weak)	Pink	Blue

Conditions: Descending technique, Whatman No. 1 paper; system A: 1-butanol-acetic acid-water (4:1:5); system B: acetone-benzene-water (6:1:1). Spray reagents: (i) aniline-iodate reagent (1,2), 0.5 ml. aniline with 5 ml. of 1.0*N* potassium iodate and 20 ml. of 0.2*M* acetate buffer, pH 3.7; paper dried at 100° for 5 minutes; and (ii) DCQ reagent, 0.5% in ethanol; air-dried paper exposed to ammonia vapor.

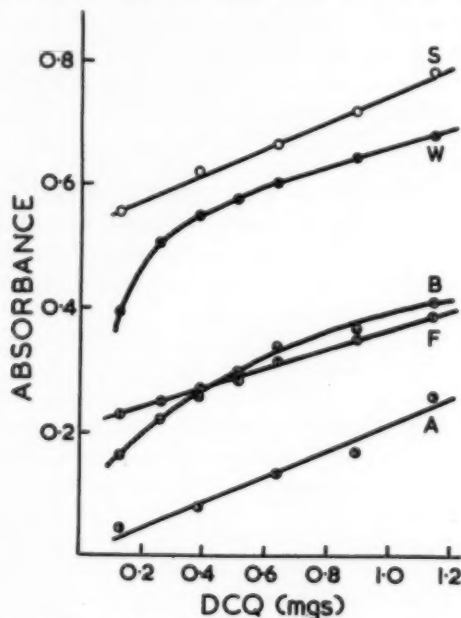


Fig. 1A. Relation between absorbance and DCQ. A, absorbances with addition of varying amounts of DCQ to mixtures of 2 ml. buffer, pH 9.4, and 1 ml. water, to which were added 2 ml. each of water (curve A), of MHQ glucoside equivalent to 48  $\mu$ g. MHQ (curve S), and of extracts from 0.3 g. whole wheat (curve W), from 0.5 g. white flour (curve F), and from 0.2 g. bran (curve B).

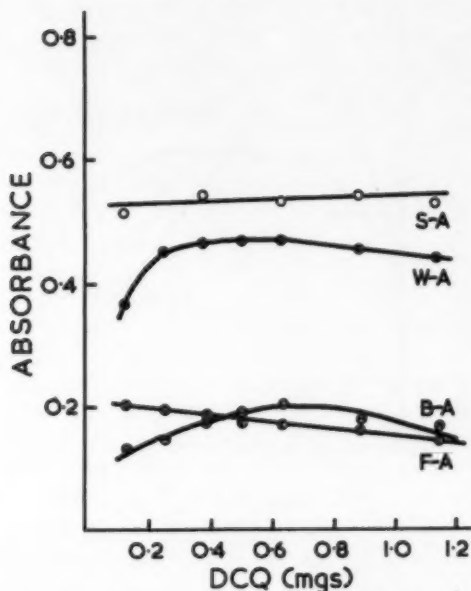


Fig. 1B. Absorbances of Fig. 1A, minus the corresponding blanks.

the low value of its  $R_F$  may indicate that it is a disaccharide derivative of MHQ. It was mainly this substance which was determined in the quantitative experiments, the one with  $R_F$  0.14 being of minor importance.

The amount of DCQ to be added in quantitative determination was established empirically. Figure 1A shows how absorbance varied with the addition of increasing amounts of DCQ to 1) water (blank, curve A), 2) standard MHQ glucoside solution (curve S), and aqueous extracts of 3) whole wheat (curve W), 4) commercial white flour (curve F), and 5) wheat bran (curve B), each at a constant level. The curves in Fig. 1B were obtained by plotting the differences between the absorbances of Fig. 1A and the blanks. They show that the apparent indophenol yields for the three extracts are at maxima at about 0.5, 0.6, and 0.15 mg. DCQ for whole-wheat, bran, and white-flour extracts, respectively. For practical application of the reaction to the analysis of wheat fractions, a constant addition of DCQ (0.5 mg.) has been adopted. The deviations of indophenol yields from the maximum obtainable are then no more than the other experimental errors in the method.

When a whole-meal flour was extracted with water, an initial in-

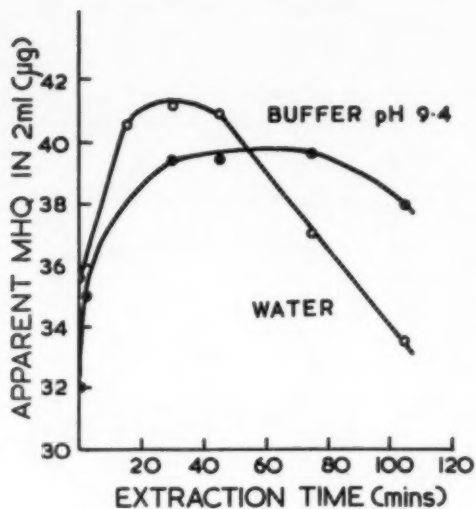


Fig. 2. Variation, with time, in apparent MHQ contents of 2 ml. of extracts from 0.3 g. whole wheat. The pH of the water extracts was 6.4 throughout the extraction period.

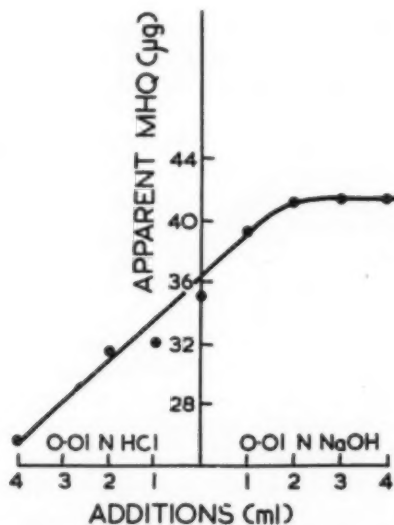


Fig. 3. Apparent MHQ contents of 2 ml. of extracts from 0.3 g. whole wheat obtained with various extractants; time of extraction, 105 minutes.

crease in apparent MHQ content was followed by a decrease, probably caused by enzymatic hydrolysis. Figure 2 illustrates this effect, and also shows how a nearly constant value was attained if buffer of pH 9.4 was used. Figure 3 shows the dependence of apparent MHQ content on pH with a long extraction time, hydrolysis being favored by acidic and minimized by alkaline extraction. Although slightly higher results could be obtained by extraction with sodium hydroxide, the use of the buffer solution eliminated one complication from the method and improved reproducibility.

### Application to Wheat Fractions

*Wheat Fractions and Extraction.* Individual grains of a sample of English wheat, variety Atle, were halved by cutting them equatorially with a razor blade; the beard and germ ends were collected separately and then milled as described below.

Whole grains of Manitoba wheat were degermed by exposing them to attack by adult *Tribolium confusum* beetles; the degermed grains

TABLE II  
MHQ CONTENTS OF FRACTIONS MILLED FROM THE SEPARATED BEARD AND GERM HALVES OF ENGLISH WHEAT, VARIETY ATLE

SAMPLE	PROPORTION OF GRAIN	MHQ VALUES		PROPORTION OF TOTAL MHQ <sup>a</sup> PRESENT
		%	$\mu\text{g/g}$	
Beard-end fractions				
Flour	40.2	12.5	500	3.4
Semolina reduction				
overtails	4.7	140	650	4.5
Other fine offal	2.5	90	250	1.7
Bran	7.9	235	1,850	12.7
Beard ends (by addition)	55.3	59	3,250	22.3
Germ-end fractions				
Flour	30.4	85	2,600	17.9
Semolina reduction				
overtails	8.9	730	6,500	44.7
Other fine offal	2.5	540	1,350	9.3
Bran	2.9	295	855	5.8
Germ ends (by addition)	44.7	250	11,300	77.7
Determined separately				
Beard ends	55.3	60	3,300	22.7
Germ ends	44.7	240	10,700	73.3
Whole grain	100	152	15,200	104
Total found in 100 g. of fractions		14,550 $\mu\text{g}$ .		
Total found in 100 g. of unmilled half-grains		14,000		
Total found in 100 g. of whole grain		15,200		

<sup>a</sup> Based on the MHQ in 100 g. of fractions.

and the intact grains from the same sample were separately milled.<sup>2</sup>

Wheat samples were milled to 65–70% extraction under standard conditions on the MIAG laboratory mill with four breaks followed by three reductions of middlings ("fine first middlings"), four of semolina ("sizings"), and three of dunst ("small middlings"). For convenience, the fourteen flour streams were combined into four groups, namely: break flour, middlings reduction flour, semolina reduction flour, and dunst reduction flour. The bran and the offal removed during the reduction of semolina were also examined. Before whole-wheat samples and coarse fractions were extracted, they were ground on a Wiley laboratory mill, to pass through a 40-mesh screen. The samples were suspended in 6 ml. of buffer, pH 9.4, shaken vigorously from time to time during 1.0–1.5 hours, and then centrifuged. The weight to be extracted was chosen so as to give an MHQ yield of 5–50  $\mu$ g. in a 2-ml. aliquot of the supernatant. Each determination was repeated on another day.

TABLE III  
MHQ AND THIAMINE CONTENTS OF MILLING FRACTIONS FROM NORMAL AND  
DEGERMED MANITOBA WHEAT

SAMPLE	MHQ		THIAMINE	
	Degermed	Normal	Degermed	Normal
	$\mu$ g/g		$\mu$ g/g	
Break flour	14.9	21.6	0.50	0.72
Middlings reduction flours	17.6	30.3	0.44	0.97
Semolina reduction flours	16.3	29.1	0.31	0.94
Dunst reduction flours	...	28.2	0.25	0.81
Coarse bran	290	365	4.75	6.56
Overtails of semolina reduction	165	695	2.53	15.38
Wholemeal	54	170	1.31	3.88

<sup>a</sup> Sample lost.

Table II summarizes the milling yields from the isolated beard-end and germ-end portions of Atle wheat and gives the MHQ contents of the fractions. The values for total MHQ content found by three ways are in satisfactory agreement.

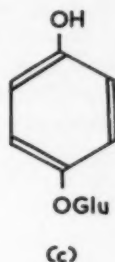
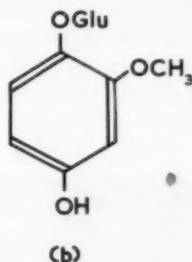
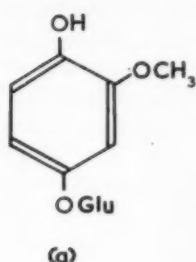
Table III gives the MHQ values of milling fractions from whole and degermed Manitoba wheat.

### Discussion

To give blue reaction products, the DCQ reagent 2:6-dichloroqui-

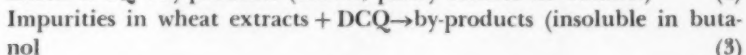
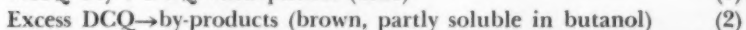
<sup>2</sup> The milling and degerming processes were carried out by D. J. Stevens, whose paper, "The contribution of the germ to the oil content of white flour," has been submitted to CEREAL CHEMISTRY.

none-4-chloroimide usually attacks only those phenols with the 4-position unsubstituted (7), and this reaction has indeed been used as a diagnostic test for such a grouping in pyridoxine (11). The reaction with MHQ glucoside (formula a or b) is therefore unusual in that the 4-position is occupied. Other examples of this type are provided



by the reaction with arbutin (formula c) and with 4-chlorophenol (5). The reaction product from the latter is believed to be the same as that from phenol (5) after elimination of the 4-substituent. The reaction with the glucoside of the two hydroquinones probably takes a similar course, since there is complete transference of the indophenols to the nonaqueous phase when their aqueous solutions are extracted with 1-butanol; this suggests that the hydrophilic sugar moiety has been removed.

It is evident that more than one reaction occurs when DCQ is mixed with wheat extracts. A qualitative explanation for the shape of the curves in Figs. 1A and 1B is given by postulation of the following reactions:



Reactions 1 and 3 alone occur in the experiments with standard MHQ glucoside solutions, and an explanation of the linearity and parallelism of curves S and A (Fig. 1A) may be found by assuming that (2) is slow compared to (1), so that they are noncompetitive. In the experiments with wheat extracts it may be assumed that reaction (3) is intermediate in speed and is competing with both (1) and (2), and that competition with (1) is predominant in the rise of curves W-A and B-A and with (2) in the more gradual fall after the maximum curve in W-A and F-A. However, an additional reason for the shape

of curves W-A and F-A may be that the brown material responsible for the normal blank is partially adsorbed on to the protein precipitated when the solutions are shaken with butanol.

It is noteworthy that as much as 22% of the apparent MHQ in Atle wheat was found in the beard halves; most of it was in the branny parts. This finding is in disagreement with the work of Bungenberg de Jong *et al.* (2) who found it only in the germ; therefore, the direct estimation of germ content in a wheat product from its MHQ value is impossible because of the contribution of MHQ from the bran and, possibly, the endosperm. The previous failure to detect it in bran may perhaps be due to the relative disparity of MHQ concentrations in bran



Fig. 4. Section of wheat grain stained with the DCQ reagent spray described in Table I. Magnification, approx. 16 diameters. Photographed in sodium light.

and germ. Calculation from the results in Table II gives a rough estimate of 3500  $\mu\text{g/g}$  for the concentration of MHQ in the germ of the English wheat, assuming that the nongerm portion of the germ ends has the same MHQ concentration as the beard ends; a similar calculation from the results in Table III gives a value of 4700  $\mu\text{g/g}$  for Manitoba wheat germ. In both samples the bran value is about one-fifteenth of the germ value. The germ provides about 60 and 70%, respectively, of the total MHQ in these two samples of wheat.

Inspection of Table III discloses a marked parallelism between the MHQ and thiamine values in milling fractions from both degermed and normal wheat. This suggests that these substances may be located in the same or in closely associated regions of the grain; the principal locations for thiamine are known to be the scutellum part of the germ and the aleurone layer adjacent to the bran (8). The photograph (Fig. 4) of a section of wheat stained by DCQ supports the suggestion, though there seems to be a substantial amount in the other parts of the germ.

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# FLOUR GRANULARITY AND COOKIE QUALITY

## I. Effect of Wheat Variety on Sieve Fraction Properties<sup>1</sup>

WILLIAM T. YAMAZAKI<sup>2</sup>

### ABSTRACT

Analytical and baking data for coarse and fine fractions obtained by sifting straight grade flours from several hard and soft wheat varieties over a 325-mesh sieve indicated a separation not only of particles by size but also of chemical composition and sugar-snap cookie-baking potentialities evaluated in terms of spread. For the harder varieties, the protein contents of the two fractions did not differ greatly, and the ash contents of the fine flours were higher than those of the coarse. Cookie quality of the two fractions was about equal, both poor. The fine flours from the soft wheat varieties, on the other hand, were lower in protein and ash contents and baked larger cookies than their coarse counterparts.

When coarse and fine fractions were blended, the resulting flours baked cookies larger than would be expected from a calculation of the weighted mean diameters of the fractions. This augmentation of spread, called the "interaction effect," had its maximum value when approximately equal quantities of coarse and fine fractions were present.

Few studies have been reported on the relation between flour particle size and cookie quality. One of these is by Wichser and Shellenberger (3), who related soft wheat flour particle size to cookie characteristics. For the flour they used, the fraction constituting the smallest particles baked cookies with the largest spread and with superior top grain. The present paper reports on a study of granulation in soft wheats, with emphasis on varietal differences in properties of flours and their sieve fractions associated with granularity.

### Materials and Methods

Experimentally milled straight grade flours of pure varieties were used for the greater part of the study. Descriptions of wheats and flours used for each experiment are given with results obtained therefrom.

Flours were separated into coarse and fine fractions by sieving on an 18 by 18-in., 325-mesh phosphor bronze sieve in a gyrator sifter box while brushing the stock lightly to shorten the time required. Replicate runs gave results which checked within 1% of the yield of fine flours.

Standard analytical procedures were followed for moisture, pro-

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tein, and ash, and results are presented on a 14% moisture basis. Alkaline water retention capacity values were obtained following the procedure of Yamazaki (4). Sugar-snap cookies were baked by the method outlined by Finney, Morris, and Yamazaki (2), and the data are expressed as the sum of diameters of two cookies.

### Results

Preliminary experiments indicated that the sieving operation was completely reversible, since blends of coarse and fines reconstituted in the original ratios baked cookies identical with those obtained from the whole flours.

*Protein Series of Three Varieties.* For the first study, three variety-types were used: Kharkof-Purkof, a hard wheat blend of the two varieties which had coarse granulation and produced poor cookies;

TABLE I  
ANALYTICAL AND BAKING DATA FOR FLOURS AND THEIR SIEVE FRACTIONS USED IN THE  
PROTEIN SERIES SIEVING EXPERIMENT<sup>a</sup>

VARIETY	PROTEIN LEVEL	GRANU- LATION	YIELD <sup>b</sup>	PROTEIN	ASH	AWRC	COOKIE SPREAD
			%	%	%	%	cm
Kh.-Purkof	High	Coarse	76	12.9	0.39	59.7	16.0
		Original		12.6	.42	58.3	16.4
		Fine	24	11.2	.50	58.2	15.6
	Med.	Coarse	74	10.6	.41	58.4	16.2
		Original		10.2	.43	57.0	16.8
		Fine	26	9.0	.49	58.1	16.2
	Low	Coarse	73	8.3	.39	56.8	16.0
		Original		8.2	.42	56.3	16.7
		Fine	27	7.5	0.50	60.7	16.3
Fairfield	High	Coarse	48	15.5	0.44	60.2	14.9
		Original		12.6	.42	49.0	17.2
		Fine	52	9.7	.39	52.4	17.9
	Med.	Coarse	45	11.9	.46	59.2	15.5
		Original		9.8	.42	47.5	17.7
		Fine	55	8.0	.39	52.7	18.2
	Low	Coarse	46	8.6	.46	56.4	15.9
		Original		7.4	.41	...	...
		Fine	54	6.3	0.38	53.3	18.2
Blackhawk	High	Coarse	54	17.4	0.36	56.0	16.2
		Original		14.3	.36	45.4	17.8
		Fine	46	10.8	.36	54.5	18.3
	Med.	Coarse	60	12.7	.39	52.1	17.0
		Original		11.3	.40	45.9	18.0
		Fine	40	9.3	.42	52.0	17.9
	Low	Coarse	65	9.1	.38	46.8	17.5
		Original		8.3	.39	45.5	18.0
		Fine	35	6.6	0.39	50.9	18.2
L.S.D. 0.05						0.36	
L.S.D. 0.01						0.48	

<sup>a</sup> 14% moisture basis.

<sup>b</sup> Based on separation on a 325-mesh sieve.

Fairfield, a soft wheat having very fine granulation and fair baking quality; and Blackhawk, a soft red winter wheat of fairly coarse granulation but capable of producing excellent cookies. Composites of each variety were made from existing flour samples milled from grain of diverse origin, such that within each variety there were blends at three protein levels. One objective of this experiment was to study the effect of protein content on granulation and baking quality for each variety, and a second was to make blends in which there would be for each variety a coarse and a fine flour at a uniform protein content, which eliminated this factor as a variable but allowed comparison of the analytical and baking results of the corresponding fractions of the several varieties. Analytical and baking data for the flours and their sieve fractions are presented in Table I.

Alkaline water retention capacity values have been included since they correlate highly with cookie spread for whole flours; the lower values indicate greater spread (4). With flour fractions, the correlation between these variables was lower, although within each granulation series the relationship was still good.

While the trends in ash and protein content changes when flours are sieved are readily evident in the table, such is not the case with cookie spread. Therefore, the effects of variety, protein level, and granulation on cookie spread were analyzed. The data for original flours were incorporated in the analysis, since the flours were considered to have an intermediate granularity.

The variety  $\times$  granulation  $\times$  protein level interaction was highly significant. The variety  $\times$  granulation interaction appeared to make the most important contribution to the second-order interaction,

TABLE II  
ANALYTICAL AND BAKING DATA FOR UNIFORM PROTEIN COARSE AND FINE  
FRACTIONS OF THREE VARIETIES<sup>a</sup>

VARIETY	GRANU- LATION	WEIGHTED MEAN YIELD <sup>b</sup>	PROTEIN	ASH	AWRC	COOKIE SPREAD
		%	%	%	%	cm
Kh.-Purkof	Coarse	74	9.0	0.40	56.9	16.3
	Fine	26	9.0	0.49	58.1	16.2
Fairfield	Coarse	46	9.0	0.46	57.1	16.0
	Fine	54	9.0	0.39	53.7	18.0
Blackhawk	Coarse	62	9.1	0.38	46.8	17.5
	Fine	38	9.0	0.42	50.7	18.0
L.S.D. 0.05						0.19
L.S.D. 0.01						0.28

<sup>a</sup> 14% moisture basis.

<sup>b</sup> Based on separation on a 325-mesh sieve.

however. The variety  $\times$  granulation interaction reflects the differential effect of granulation on the several varieties.

Analytical and baking data for the uniform protein composites prepared of a coarse and a fine fraction for each variety are presented in Table II. This table has been included to indicate the trends in ash and cookie-spread values of the fractions when flours differing in granularity were sieved through a 325-mesh sieve and the resulting fractions adjusted to a uniform protein level.

Analysis of variance of the duplicated cookie-spread data for the uniform protein samples again showed a highly significant F value for the variety  $\times$  granulation interaction.

TABLE III  
ANALYTICAL AND BAKING DATA FOR FLOURS AND THEIR SIEVE FRACTIONS USED IN THE VARIETY SERIES SIEVING EXPERIMENT<sup>a</sup>

VARIETY	GRANULATION	YIELD <sup>b</sup>	PROTEIN	ASH	COOKIE SPREAD
		%	%	%	CM
Kharkof	Coarse	78	9.5	0.39	16.0
	Original		9.2		16.7
Purkof	Fine	22	8.5	0.52	16.2
	Coarse	73	8.9	0.41	16.2
	Original		8.4		16.7
	Fine	27	8.1	0.46	16.3
Minturki	Coarse	65	10.4	0.40	16.8
	Original		9.6		17.5
	Fine	35	8.2	0.44	17.5
	Coarse	77	8.8	0.44	16.6
Kawvale	Original		8.5		16.8
	Fine	23	8.3	0.59	15.9
Clarkan	Coarse	42	11.0	0.45	16.0
	Original		9.0		18.0
	Fine	58	7.7	0.35	18.0
	Coarse	41	11.0	0.45	15.1
Trumbull	Original		8.8		17.4
	Fine	59	7.5	0.36	18.2
Fairfield	Coarse	36	9.6	0.49	15.5
	Original		7.9		17.9
	Fine	64	7.1	0.37	18.2
	Coarse	42	9.7	0.45	16.2
Thorne	Original		8.0		17.8
	Fine	58	7.0	0.37	18.2
Wabash	Coarse	49	9.9	0.41	16.6
	Original		8.3		18.4
	Fine	51	6.9	0.35	18.8
	Coarse	59	11.4	0.37	17.3
Blackhawk	Original		10.0		18.3
	Fine	41	8.3	0.37	17.9
Am. Banner	Coarse	48	9.9	0.46	16.2
	Original		8.6		17.9
	Fine	52	7.5	0.39	18.4
				L.S.D. 0.05	0.32
				L.S.D. 0.01	0.43

<sup>a</sup> 14% moisture basis.

<sup>b</sup> Based on separation on a 325-mesh sieve.

*Variety Series.* Because variety appeared to be an important factor in granulation, as shown in Table II, data were obtained on a series of flours from wheats grown under identical conditions. Experimentally milled straight-grade flours of eleven pure varieties were chosen, representing a range in type from hard red winter to soft white winter. Table III presents the analytical and baking data on the original flours and their sieve fractions.

The original cookie-spread data were combined and the results analyzed statistically. Again the variety  $\times$  granulation interaction was highly significant.

*Variety Series-Interaction Effect.* Early in the experimentation it was observed that when combinations of coarse and fine flours were

TABLE IV  
BAKING DATA FOR SEVERAL BLENDS OF COARSE AND FINE FRACTIONS

VARIETY	COOKIE SPREAD			
	All-Fine	$\frac{2}{3}$ Fine, $\frac{1}{3}$ Coarse	$\frac{1}{2}$ Fine, $\frac{1}{2}$ Coarse	All-Coarse
	cm	cm	cm	cm
Kharkof	16.2	16.5	16.5	16.0
Purkof	16.3	16.9	16.8	16.2
Minturki	17.5	17.7	17.7	16.8
Kawvale	15.9	16.6	16.6	16.6
Clarkan	17.8	18.2	17.2	16.0
Trumbull	18.2	17.6	16.8	15.1
Fairfield	18.2	17.9	16.9	15.5
Thorne	18.2	18.0	17.2	16.2
Wabash	18.8	18.3	17.8	16.6
Blackhawk	17.9	18.3	18.0	17.3
Am. Banner	18.4	18.3	17.4	16.2
			L.S.D. 0.05	0.31
			L.S.D. 0.01	0.41

made, the cookie spreads of the resulting flours were larger than the weighted means of spread of the fractions. Using the variety series of samples, this interaction effect was studied further. For each variety, flour blends were made of coarse and fine fractions in the ratios of 2 to 1 and 1 to 2, and cookies were baked. Table IV presents baking data for these blends as well as for the fractions themselves.

The original duplicated baking data showed a highly significant variety  $\times$  granulation interaction.

The presence of four granulation levels permitted the further subdivision of the granulation effect into linear and quadratic components within each variety. Table V presents the results of such an analysis.

As can be seen above, the quadratic components for all varieties

TABLE V  
ANALYSIS OF VARIANCE OF COOKIE SPREAD FOR LINEAR AND QUADRATIC COMPONENTS  
OF THE GRANULATION EFFECT

VARIETY	d.f.	M.S. LINEAR	M.S. QUADRATIC	F LINEAR	F QUADRATIC
Kharkof	3	0.05110	0.30030	2.23	13.11**
Purkof	3	0.01295	0.73205	0.57	31.97**
Minturki	3	0.41820	0.53560	18.26**	23.39**
Kawvale	3	0.35910	0.23460	15.68**	10.24**
Clarkan	3	4.33620	1.22460	189.35**	53.47**
Trumbull	3	10.34290	0.47045	451.65**	20.55**
Fairfield	3	8.23555	0.69030	359.63**	30.14**
Thorne	3	4.68540	0.30710	204.60**	13.45**
Wabash	3	4.67855	0.23805	204.31**	10.40**
Blackhawk	3	0.43055	0.58860	18.80**	25.70**
Am. Banner	3	5.46860	0.51510	238.80**	22.49**
Error	44	0.02292			

were highly significant. This result denoted a definite curvilinear relationship between granulation and cookie spread for each variety tested. The deviation from linearity has been termed the "interaction effect."

Cookie-spread values were therefore interpolated from the baking data for other ratios of coarse and fine. The weighted mean cookie-spread values were then calculated for the same ratios, and the difference in spread between these mean and interpolated values was attributed to the "interaction effect," or the augmentation of spread due to the presence in the flour of particles which differ considerably in size. The average maximum interaction was found to take place when approximately 48% coarse flour was present in the blend. Table VI presents granularity and interaction effect on the cookie spread

TABLE VI  
GRANULARITY AND INTERACTION EFFECT ON COOKIE SPREAD OF FLOURS OF  
ELEVEN WHEAT VARIETIES

VARIETY	GRANULARITY AT POINT OF MAXIMUM INTERACTION EFFECT	EXTENT OF MAXIMUM INTERACTION
	% coarse	cm
Kharkof	55	0.6
Purkof	50	0.6
Minturki	55	0.5
Clarkan	40	0.9
Trumbull	60	0.6
Fairfield	45	0.6
Thorne	35	0.5
Wabash	50	0.5
Blackhawk	50	0.7
Am. Banner	40	0.6
Mean	48	0.597 $\pm$ 0.124

of the samples tested. The last column refers to the increase in spread over that expected, assuming the absence of interaction. For example, the fine fraction of Fairfield baked cookies with a spread of 18.2 cm., and the corresponding coarse fraction, a spread of 15.5 cm. If equal quantities of each were blended, it would be expected that the flour would bake cookies with a spread of 16.9 cm. The interpolated value, based on actual cookies obtained at other ratios, was 17.5 cm. The difference between 16.9 and 17.5 cm., or 0.6 cm., was ascribed to the interaction effect. Similar results were obtained when fractions of different varieties were interblended.

On the basis of the above results, an idealized table of interaction effect was prepared as follows:

Percent coarse	100	90	80	70	60	50	40	30	20	10	0
Interaction effect (cm.)	0.0	0.2	0.4	0.5	0.5	0.6	0.6	0.5	0.4	0.2	0.0

The efficiency of this procedure in predicting spread was then tested on several flours for which cookie data were already available for coarse and fine fractions, as well as for the original flour. These results are presented in Table VII.

TABLE VII  
COMPARISON OF CALCULATED AND ACTUAL COOKIE SPREADS OF CERTAIN FLOURS

SAMPLE NUMBER	COARSE FLOUR YIELD <sup>a</sup>	WEIGHTED MEAN SPREAD	INTERACTION EFFECT	ADJUSTED COOKIE SPREAD	ACTUAL SPREAD
	%	cm	cm	cm	cm
1	80	15.5	0.4	15.9	15.8
2	50	17.2	0.6	17.8	17.7
3	35	16.2	0.6	16.8	16.8
4	80	15.9	0.4	16.3	16.4
5	80	16.2	0.4	16.6	16.8
6	80	16.1	0.4	16.5	16.6
7	50	16.5	0.6	17.1	17.2
8	45	17.0	0.6	17.6	17.6
9	55	17.1	0.5	17.6	17.8
10	60	17.4	0.5	17.9	18.0
11	65	17.7	0.5	18.2	18.0

<sup>a</sup> Based on separation on a 325-mesh sieve.

As may be seen from a comparison of the data, there was good agreement between expected and actual cookie spreads when the interaction effect was taken into consideration.

### Discussion

Increases in protein content within a variety did not necessarily increase the granularity of flours as determined by sieving. Fine flour yields from Table I gave variety and protein level means as follows:

Variety Means		Protein Level Means	
	%		%
Kharkof-Purkof	25.67	High	40.67
Fairfield	53.67	Medium	40.33
Blackhawk	40.33	Low	38.67
L.S.D. 0.05	6.37	Differences are nonsignificant	
L.S.D. 0.01	10.56		

This result tended to confirm the contention of Berg-(1) that granulation is a varietal factor rather than a protein quantity characteristic, for these samples at least.

According to their yield of fine flour, the several varieties tested may be ranked in order of increasing "softness" as follows: Kharkof, Kawvale, Purkof, Blackhawk, Minturki, Wabash, American Banner, Thorne and Clarkan (tied), Trumbull, and Fairfield. However, this is not the ranking of cookie quality (see Table IV) which shows Wabash and Blackhawk flours to be superior in diameter. This result is an indication that inherent varietal characteristics other than endosperm-fracturing properties are the more important consideration.

The analysis of the coarse and fine fractions followed certain trends which were associated with granularity. Thus, the difference in protein content between the two fractions of a sample increased with

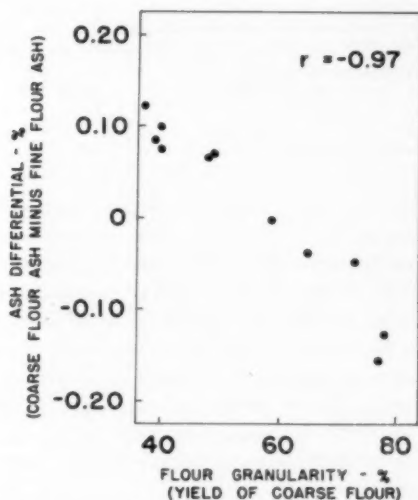


Fig. 1. Scattergram showing relationship between flour granularity (as expressed by yield of coarse flour over a 325-mesh sieve) and ash difference (coarse flour ash in percent minus fine flour ash in percent) for flours from eleven wheat varieties.

increasing "softness." In the case of the ash data, the relationship between varietal granularity and differential in analysis is more striking and is presented as a scattergram in Fig. 1. For the Kharkof-Purkof samples of the three-variety study, the coarse fractions had less ash than the fines, the differences being rather uniform regardless of initial protein content. The Fairfield fractions had ash contents which were in accord with expected trends, with the fines being lower in mineral constituents. Blackhawk differed from the other two varieties in that the fractions showed small differences in ash contents.

The "harder" varieties produced coarse and fine fractions which baked cookies that were similar in diameter. On the other hand, larger cookies were baked by the fine fraction of the "softer" varieties than the corresponding coarse fractions; the spread difference, in general, was in line with granularity ranking.

These relationships are summarized in Table VIII, which presents correlation coefficients of a nature similar to that calculated for the association between flour granularity (or coarse flour yield) and ash difference between fine and coarse sieve fractions.

TABLE VIII  
CORRELATION COEFFICIENTS FOR RELATIONSHIPS AMONG COARSE FLOUR YIELD AND EXTENTS OF PROTEIN, ASH, AND COOKIE SPREAD DIFFERENCES BETWEEN COARSE AND FINE FLOURS OF ELEVEN VARIETIES

RELATIONSHIP	n	r
Coarse flour yield vs. protein difference	11	-0.873
Coarse flour yield vs. cookie-spread difference	11	-0.944
Coarse flour yield vs. ash difference	11	-0.978
Cookie spread difference vs. ash difference	11	0.931
Cookie spread difference vs. protein difference	11	0.833

The table indicates that when the yield of coarse flour is high (that is, when the wheat is "harder"), the difference in protein, ash, and cookie spread between the coarse and fine flours is less than if the wheat is "softer" or has a lower yield of coarse flour.

In the statistical analysis of cookie-diameter data, it was found that in each case there was a significant variety  $\times$  granulation effect, where granulation was expressed in terms of all-coarse, intermediate, and all-fine fractions. These results indicate that the effect of granularity on cookie diameter is a function of the variety used, and that it reflects the extent of differences in cookie spread between the coarse and fine fractions.

If the varieties are analyzed individually to remove the interaction, it then becomes possible to determine whether the change in cookie

diameter is a linear or quadratic function of granulation. The fact that the quadratic mean squares for all varieties are highly significant (Table VI) demonstrates the applicability of a curvilinear relationship. This curvilinearity is the basis of the "interaction effect" previously described. For the experimentally milled flours used, the maximum augmentation appeared when approximately equal quantities of coarse and fine fractions were present. Therefore, it would seem that flours which are either very coarse or very fine would not have as great an advantage of the interaction effect. The magnitude of this effect is, however, not great compared to the contribution to flour cookie quality of the qualities of the coarse and fine fractions themselves, attributes which are varietal characteristics.

#### Acknowledgment

The author wishes to thank Dr. C. R. Weaver, Statistician of the Ohio Agricultural Experiment Station, for statistical advice, and Lloyd Moser for technical assistance.

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## FLOUR GRANULARITY AND COOKIE QUALITY

### II. Effects of Changes in Granularity on Cookie Characteristics<sup>1</sup>

WILLIAM T. YAMAZAKI<sup>2</sup>

#### ABSTRACT

Changes in flour granularity brought about by variable temper in a single wheat mix did not affect significantly the sugar-snap cookie-baking potentialities of the straight grade flours milled. However, flours obtained from higher-temper millings of a soft wheat contained fine sieve fractions which were lower in ash and protein contents, and produced larger cookies than did their coarse fractions. The protein and ash contents of the coarse fractions from millings of a hard wheat did not change appreciably, but cookie potentialities decreased somewhat with increasing temper applied to the wheat. On the other hand, the corresponding fine fractions decreased in protein and ash and increased in cookie spread.

Mill stream selection, while generally maintaining flour granularity, may result in modified cookie potentialities because of changes in quantities of flour components that affect cookie quality. Excessive reduction of flours caused deterioration of cookie spread, apparently through damage to flour.

These results support the belief that flour factors other than granularity are more important in determining cookie quality.

In a companion paper (4), various comparisons were made of fine and coarse sieve fractions obtained by passing flour over a 325-mesh sieve; straight grade experimentally milled flours produced under normal conditions of temper and roll settings were used as a starting material. Fine fractions from flours of the harder varieties were slightly lower in protein and higher in ash than the coarse fractions and produced cookies equal in spread to their coarse counterparts. In soft wheat flours, the fine fractions were considerably lower in protein and ash contents and gave superior cookies. Flour granularity as measured by yield of fine fractions appeared to be a varietal characteristic unaffected by protein content within a variety.

This paper presents data pertaining to properties of sieve fractions of flours which were modified in the mill by mill stream separation, variable temper of wheat, and selective reduction of coarse fractions.

#### Materials and Methods

The flours were obtained in the various manners described below.

In the first case, the mill streams of a normal Buhler milling of a soft wheat (Thorne) were kept separate.

<sup>1</sup> Manuscript received December 9, 1957. Cooperative investigation between the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and Department of Agronomy, Ohio Agricultural Experiment Station, Wooster, Ohio. A portion of this discussion was presented at the 40th annual meeting, St. Louis, Mo., May 1955.

<sup>2</sup> Chemist, Crops Research Division, A.R.S., U. S. Department of Agriculture, Soft Wheat Quality Laboratory, Wooster, Ohio.

For the second, a hard red winter (Pawnee) and a soft red winter (Thorne) wheat were treated to give both high and low moisture levels which produced flours differing in granularity, as demonstrated by Sandstedt and Fleming.<sup>3</sup> The moisture content of the former was adjusted to give a range from 4.6 to 20%, and that of the latter from 10 to 25%.

In the last series, three samples were used. These were the medium protein samples of a Kharkof-Purkof blend, Fairfield, and Blackhawk discussed in the companion paper (4) under "Protein Series of Three Varieties." The coarse sieve fraction for each sample was reduced on the smooth rolls of a laboratory Allis-Chalmers mill to give approximately 60% yield of fine flour.

After analysis, blends of these fractions were made in their original ratios with the fine fractions previously obtained, both before and after the reduction process.

In all cases, the flours were sieved, analyzed, and baked as previously described (4).

### Results

*Mill Stream and Granularity.* Table I presents data bearing on the mill streams of Thorne wheat, together with similar data on the sieve fractions obtained therefrom. For a parallel milling, the straight grade flour produced cookies with a spread of 17.3 cm.

An analysis of variance of cookie spread using duplicated data indicated that, while mill stream and granulation both had significant F values, the interaction  $MS \times G$  was also highly significant.

*Variable Temper and Granularity.* Some difficulty was encountered in obtaining the desired yields in milling wheat at the extreme levels. In the case of Thorne, lowered yields resulted from drying the grain prior to milling and at the extremely high tempers (17.5 and 20% moisture). In addition, bolting troubles were the rule for the latter samples. Similar difficulties were met in milling the Pawnee wheat. These shortcomings were reflected by the yield and analytical data in Table II.

Table III gives the yield and analytical data for the sieve fractions obtained from the flours enumerated in the previous table.

Results for the samples receiving the extreme tempers, while perhaps not strictly comparable to those obtained under more moderate conditions, are nevertheless included because they appeared to show certain trends.

<sup>3</sup> Sandstedt, R. M., and Fleming, J. C. A motion photomicrographic study of the disintegration of the wheat kernel endosperm when subjected to pressure. The effects of kernel hardness and of tempering on the physical characteristics of the resulting flour. Presented at the 37th annual meeting, Dallas, Texas, April 1952.

TABLE I  
YIELD, ANALYTICAL, AND BAKING DATA FOR FLOURS AND COARSE AND FINE FRACTIONS  
OF THORNE MILL STREAMS<sup>a</sup>

FLOUR	YIELD OF FLOUR <sup>b</sup>	PROTEIN	ASH	AWRC	COOKIE DIAMETER
	%	%	%	%	cm
First break	10.6	8.1	0.28	50.8	17.8
Second break	12.8	10.5	.31	49.6	17.2
Third break	4.3	13.0	.68	52.8	16.2
First reduction	37.7	9.3	.33	47.7	17.3
Second reduction	18.1	9.8	.43	51.6	16.6
Third reduction	10.2	10.6	0.73	57.5	15.9
SIFTING YIELD <sup>c</sup>					
	%				
First break over	34.0	10.5	0.33	52.7	16.3
First break through	66.0	7.1	.28	56.0	17.8
Second break over	33.4	13.8	.35	58.1	15.7
Second break through	66.6	8.8	.31	58.6	17.5
Third break over	37.6	16.7	.78	60.3	15.0
Third break through	62.4	10.8	.62	61.3	16.8
First reduction over	52.9	10.8	.34	54.1	16.2
First reduction through	47.1	8.0	.33	54.9	17.7
Second reduction over	45.5	11.5	.48	57.3	14.8
Second reduction through	54.5	8.6	.39	57.0	17.4
Third reduction over	37.9	13.3	.99	65.6	14.0
Third reduction through	62.1	9.2	0.56	58.1	17.2
				L.S.D. 0.05	0.42
				L.S.D. 0.01	0.57

<sup>a</sup> 14% moisture basis.

<sup>b</sup> Rebolt flour yield (from bran and shorts) was 6.3%.

<sup>c</sup> Based on separation on a 325-mesh sieve.

TABLE II  
YIELD, ANALYTICAL, AND BAKING DATA FOR FLOURS OBTAINED IN THE  
VARIABLE TEMPER SERIES<sup>a</sup>

VARIETY	TEMPER	MILLING YIELD	PROTEIN	ASH	COOKIE DIAMETER
	%	%	%	%	cm
Thorne	4.6	59.1	10.1	0.58	17.8 <sup>b</sup>
	10.0	65.8	9.8	0.48	17.4
	15.0	65.7	9.5	0.35	17.3
	17.5	62.0	9.4	0.32	17.5
	20.0	60.6	9.0	0.35	17.2 <sup>b</sup>
Pawnee	10.0	68.2	11.8	0.56	16.8
	15.0	68.1	11.8	0.44	16.7
	20.0	60.5	11.4	0.42	16.5
	22.5	58.1	11.1	0.49	16.2 <sup>b</sup>
	25.0	51.0	10.8	0.52	16.3 <sup>b</sup>

<sup>a</sup> 14% moisture basis.

<sup>b</sup> Not included in statistical analysis.

TABLE III  
YIELD, ANALYTICAL, AND BAKING DATA FOR COARSE AND FINE FRACTIONS OF FLOURS  
OBTAINED IN THE VARIABLE TEMPER SERIES<sup>a</sup>

VARIETY	TEMPER	FRACTION	SIFTING YIELD <sup>b</sup>	PROTEIN	ASH	COOKIE DIAMETER
	%		%	%	%	cm
Thorne	4.6	Over	64.5	10.6	0.56	17.0 <sup>c</sup>
		Through	35.5	9.0	.56	16.8 <sup>c</sup>
	10.0	Over	63.9	10.5	.46	16.9
		Through	36.1	8.7	.48	17.2
	15.0	Over	52.7	10.8	.34	16.1
		Through	47.3	8.1	.34	17.6
	17.5	Over	43.4	11.5	.33	15.7
		Through	56.6	7.6	.29	17.9
	20.0	Over	29.6	12.4	.42	14.6 <sup>c</sup>
		Through	70.4	7.7	0.31	17.9 <sup>c</sup>
Pawnee	10.0	Over	82.3	11.6	0.48	16.7
		Through	17.7	11.2	.84	15.3
	15.0	Over	83.7	12.0	.40	16.5
		Through	16.3	10.9	.59	15.2
	20.0	Over	73.7	12.1	.42	16.2
		Through	26.3	9.6	.42	15.7
	22.5	Over	65.2	12.0	.49	16.1 <sup>c</sup>
		Through	34.8	9.3	.45	16.1 <sup>c</sup>
	25.0	Over	63.2	11.8	.52	15.9 <sup>c</sup>
		Through	36.8	9.2	0.47	16.0 <sup>c</sup>
L.S.D. 0.05						0.43

<sup>a</sup> 14% moisture basis.

<sup>b</sup> Based on separation on a 325-mesh sieve.

<sup>c</sup> Not included in statistical analysis.

TABLE IV  
ANALYSIS OF VARIANCE OF COOKIE SPREAD USING THE COARSE, ORIGINAL, AND FINE  
FLOUR DATA AT THREE TEMPER LEVELS AND TWO VARIETIES OF WHEAT

SOURCES OF VARIATION	d.f.	M.S.	F
Variety	1	8.1796	53.74**
Temper level	2	0.0700	0.46
Granularity	2	1.9350	12.71*
TL × G	4	0.5758	3.78
TL × V	2	0.0088	0.06
V × G	2	4.4898	29.50**
TL × G × V	4	0.1522	3.69*
Error	18	0.0412	

Table IV presents the analysis of variance of cookie spread using only the Thorne data at 10, 15, and 17.5% temper and the Pawnee data at 10, 15, and 20% temper, since the others appeared to be quite low in yield.

*Reduction of Coarse Sieve Fractions.* The pertinent data for the coarse fractions of the three flours used in this study both before and after reduction are presented in Table V, and Table VI gives the cookie diameters of the reblended samples.

TABLE V  
YIELD, ANALYTICAL, AND BAKING DATA FOR COARSE FRACTIONS AND FOR FRACTIONS  
AFTER REGRINDING THE COARSE FLOURS IN OVERGRINDING SERIES<sup>a</sup>  
(Original coarse)

FLOUR	YIELD (% of Flr)	PROTEIN	ASH	AWRC	COOKIE DIAMETER
	%	%	%	%	cm
Kh.-Purkof over	73.5	10.6	0.41	58.4	16.2
Fairfield over	45.3	11.9	0.46	59.2	15.5
Blackhawk over	60.0	12.7	0.39	52.1	17.0

(Sieve separates after regrinding)

VARIETY	NUMBER OF PASSES	SEPARATE	YIELD <sup>b</sup>	PROTEIN	ASH	AWRC	COOKIE DIAMETER
			%	%	%	%	cm
Kh.-Purkof	4	over	37.2	11.0	0.41	65.1	14.5
		through	62.8	10.3	0.42	61.1	15.2
Fairfield	2	over	38.4	12.8	0.53	72.9	13.9
		through	61.6	11.4	0.42	56.5	15.5
Blackhawk	3	over	37.3	13.9	0.46	61.7	14.9
		through	62.7	11.8	0.36	49.9	16.4

<sup>a</sup> 14% moisture basis.

<sup>b</sup> Based on separation on a 325-mesh sieve.

TABLE VI  
COOKIE DATA AND MEAN VALUES FROM NORMAL AND REGROUND COARSE FRACTIONS AND  
NORMAL FINE FLOURS REBLENDED IN THEIR ORIGINAL RATIOS

	COOKIE DIAMETER		VARIETY MEAN
	Normal	Reground	
	cm	cm	cm
Kharkof-Purkof	16.8	15.7	16.25
Fairfield	17.7	16.9	17.30
Blackhawk	18.1	16.7	17.40
Treatment mean	17.53	16.43	

L.S.D. 0.05 (Treatment mean) = 0.27 cm.

L.S.D. 0.01 (Treatment mean) = 0.40 cm.

L.S.D. 0.05 (Variety mean) = 0.33 cm.

L.S.D. 0.01 (Variety mean) = 0.49 cm.

Analysis of the duplicated cookie-spread data indicated that both the grinding treatment and variety had highly significant influences on spread and that the treatment  $\times$  variety effect was nonsignificant. Because of this nonsignificance, a direct comparison of effects of both variety and treatment can be made on the mean cookie data in Table VI.

### Discussion

In contrast to the situation (4) wherein granularity differences among flours were associated with varietal differences, attempts have been made in the present study to modify the granulation and cookie

quality characteristics within a variety or sample by several means, such as mill stream separation, variable temper, and reduction of coarse fractions.

There did not appear to be large differences in granularity of the break streams. However, the cookie spreads of these streams differed, decreasing with each succeeding break (Table I). That this effect is not due primarily to the increase in protein content has already been indicated in a companion paper (4), where it was shown that protein content differences did not result in large changes in sieve fraction yield ratio or cookie diameter. A cause of cookie quality difference of the various mill streams may lie in the quantity of purified starch tailings (3). The variations in cookie diameters for the streams confirm the findings of Garnatz, Hanson, and Lakamp (2) and indicate that, for a given sample of wheat, cookie spread for the resulting flour may be modified by stream selection.

Of the samples tested in the variable temper series, the low yields for Thorne at 4.6 and 20% and Pawnee at 22.5 and 25% temper (Table II) may bring in an effect analogous to mill stream selection. Considering the Thorne samples of 10, 15, and 17.5% temper, the whole flours do not differ appreciably in cookie potentialities. For Pawnee, the yield at 20% temper is low, but other experiments at 17.5% have indicated good yield.

The trends in ash, protein, and cookie diameter differentials for the coarse and fine as indicated in Table III are of interest. Within the Thorne series, for example, the protein content difference at 4.6% temper is 1.6% (10.6% for coarse, 9.0% for fine), whereas at 20% temper the range is 4.7% (12.4% for coarse, 7.7% for fine). For analogous Pawnee samples, the differences are 0.4 and 2.6%. Similar trends are noted for ash and cookie diameter differentials between fractions. These differentials are very similar to those noted when granularity differed because of varietal differences under normal tempering conditions (4). Thus, it appears that these analytical and baking characteristics for fractions are due to granulation differences rather than varietal.

The reduction of coarse fractions results in definite losses in cookie-baking potentialities, as data in Tables V and VI show. Since all of the flour fractions are accounted for, it appears that the decrease in spread was the direct result of regrinding.

On the basis of the results presented here and elsewhere (3,4), it appears that cookie-baking potentialities of a flour are dependent on qualitative and quantitative factors. The qualitative factor is a function of variety and appears to depend to some extent upon the purified

starch tailings content of the flour, although the possible effect of other constituents, such as gluten, cannot be ignored. Excessive reduction of flour, which results in smaller cookies, may have caused an alteration in the quality factor such as mechanical injury to starch, since the flour quantity was not changed during the grinding process. Under the category of qualitative effect also may be placed the so-called "interaction effect" (4), or the augmentation of cookie spread of a flour due to the presence of particles differing in granularity, the magnitude of which depends on the ratio of coarse and fine flours.

In addition, changes in granularity of flours resulting from the milling of a wheat mix tempered to various levels are not associated with significant cookie diameter changes within reasonable limits of moisture contents. Since the qualitative or variety factor remains uniform under these conditions, it seems that granularity in itself is not a dominating influence. When the qualitative factor is under discussion, the assumption is made that straight grade flours of normal yield are being tested.

The quantity factor in a wheat may be changed by such action as mill stream selection, whereby stocks containing disproportionate quantities of desirable or undesirable components are retained, reduced, or omitted. Milling equipment may bring about what amounts to stream selection by differences in bran cleanup or choice of sieves. In this connection the paper by Finney, Heizer, Shellenberger, Bode, and Yamazaki (1) is of interest. Using the same wheat mixes, they found that commercially milled flours produced the poorest cookies, Buhler experimentally milled flours the next, and the Hobart-milled (modified coffee grinder) flours made the largest cookies. If one considers the relative efficiency of these milling methods, it appears that their results are generally in line with the present discussion, although the presence of both the quality and quantity factors in their results complicate somewhat the interpretation of data.

Thus, it seems probable that when normally milled straight grade flours are being compared for cookie quality, the flour attributes associated with variety are the principal factors concerned and that particle size in itself is not so important. There may be cases when granulation would appear to be the main cause of quality differences, but, in such an event, it would be desirable to determine whether such a granulation change was brought about by agents which would cause alterations in the quantity and/or quality factor.

#### Acknowledgments

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## THE APPLICATION OF HEAT IN THE TESTING OF FLOURS FOR COOKIE QUALITY<sup>1</sup>

WILLIAM T. YAMAZAKI<sup>2</sup>

### ABSTRACT

Procedures are described for the modification of physicochemical testing of soft wheat flour by the application of heat. Results of the heating mixograph, heated alkaline water retention capacity, and viscograph tests indicate that straight-grade, experimentally milled flours of varieties which bake poorer sugar-snap cookies (smaller diameters) show viscosity increases earlier and at lower temperatures of the heating process. These differences were not attributable to varietal differences in rates of heat penetration or of losses in volatile ingredients.

It has been customary to estimate the baking quality of soft wheat flours by subjecting them to various chemical and physicochemical tests. A disadvantage of this procedure has been that the results have not always been correlated with baking quality. Among the baking tests often used for evaluating the quality of flours for pastry purposes is the cookie test (1,2,3,5). Cookie doughs are mildly alkaline, and thus a logical step in the testing program was the introduction of physicochemical procedures which would also be run in alkaline media (4,6).

One of the objectives in the development of new physicochemical

<sup>1</sup> Manuscript received December 23, 1957. Cooperative investigations between the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, and Department of Agronomy, Ohio Agricultural Experiment Station. A portion of this paper was presented at the annual meeting, San Francisco, Calif., May, 1957.

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methods is a test which would correlate highly with cookie quality and serve as a substitute for actual baking. Perhaps more important is the implication that such a test would measure a factor or factors in flours responsible for quality, and thus would open the way to a more thorough study of the mechanism of the cookie-baking process. It was believed that in physicochemical evaluation of flours, the application of heat in a mildly alkaline medium would simulate more closely conditions prevailing in the actual baking process, and hence would be more likely to reflect changes occurring in the oven. Accordingly, various physicochemical procedures were modified to include heating of a dough or flour suspension as an integral part of the test.

While this paper reports some of the results obtained in physicochemical tests of flours with the use of heat, it is intended primarily to point out some of the possibilities when these and similar methods are used.

### Materials and Methods

Four procedures were employed. The first, called the heating mixograph test, utilized a conventional National-Swanson-Working Mixograph unit modified to reduce the speed of the mixer to 25 r.p.m., by substituting a larger pulley on the planetaries. Other changes consisted of fitting a special Glas-Col heating mantle of 300-watt capacity around the mixograph bowl, and inserting an iron-constantan thermocouple through the center of the bottom of the bowl and connecting it through a cold junction to a millivolt recorder. The thermocouple extended a distance of one-half inch from the bottom, so that when a mixed cookie dough was transferred to the bowl the junction was  $\frac{1}{8}$  to  $\frac{3}{16}$  in. under the surface of the dough. As heat was applied, the dough usually rose to a maximum height of about  $1\frac{1}{2}$  in., increasing the depth of the thermocouple junction to about 1 in.

The second procedure was the determination of the alkaline water retention capacity of flours (6) at various temperatures by preheating the sodium bicarbonate solution prior to addition to flour and by holding the suspension at the desired temperature during hydration.

The use of the Brabender Viscograph constituted the third method. This instrument is similar to the Amylograph but covers a wider range of viscosity. A thermoregulator was used which increased the temperature of the sample by  $2.5^{\circ}\text{C.}$  per minute. Since cookie doughs were too viscous to be treated in this instrument, an alkaline batter was usually made which consisted of 200 g. flour, 200 ml. water, 120 g. sugar, and 1 g. sodium bicarbonate.

The fourth method involved the baking of cookies in the oven and obtaining diameter, weight loss, and internal dough temperature data on partially baked doughs. A special cookie sheet was fabricated from sheet aluminum, which measured 13 in. by 10 in. by 0.094 in. thick and which had riders running lengthwise along both sides to maintain rigidity. Cookie dough thickness was adjusted by means of movable runners along the riders. The sheet was equipped with a holder to which a thermocouple could be attached in such a way that the junction would be locked in position within the dough at a distance of  $\frac{3}{8}$  in. above the sheet. The thermocouple lead extended through a cold junction to a millivolt recorder.

Rather than two small cookies baked on a sheet, as is customary with the routine micro method in use at the Soft Wheat Quality Laboratory (3), a single large dough was cut which measured  $2\frac{3}{4}$  in. in diameter and  $\frac{5}{8}$  in. in thickness. Preliminary bakes had indicated that the diameters of cookies baked from doughs of this size correlated well with those baked by the usual micro method.

In making a test, a cookie dough was mixed as described previously (3), the bowl and contents weighed, and the dough transferred to a square of thin aluminum foil on the sheet. The dough was rolled and cut and then positioned so that the thermocouple bulb would penetrate at approximately its center to a depth of  $\frac{1}{4}$  in. The dough resi-

TABLE I  
VARIETY, CLASS, AND COOKIE-QUALITY RANKINGS OF FLOURS USED IN THIS STUDY

VARIETY	CLASS	DIAMETER	INTRINSIC COOKIE QUALITY
		cm	
Comanche (CO)	Hard red	10.4	Very poor
Purkof (P)	Semihard red	11.5	Poor
Kawvale (K)	Soft red	11.6	Poor
Trumbull (TR)	Soft red	12.0	Fair
Clarkan (CL)	Soft red	12.5	Good
Am. Banner (A)	Soft white	12.6	Good
Blackhawk (B)	Soft red	12.8	Good

due was replaced in the original mixing bowl and reweighed to obtain the initial dough weight. The entire cookie sheet assembly with dough was placed in the oven and baked for a predetermined time; at the end of that period, the sheet was taken out of the oven, thermocouple removed, and the foil with the dough placed quickly in a tared, flat can (provided with a tight lid), which was immediately weighed to ascertain the loss of volatile ingredients. Diameter measurements of these partially baked cookies were taken as soon as they cooled. With

the oven at 400°F., 16 to 17 minutes were required for the complete baking of these larger cookies.

All of the samples used were straight-grade, unbleached, experimentally milled flours from varietal composites of grain grown at several locations over three crop years. The varieties used, class of each, and cookie data obtained are presented in Table I.

Not all of the flours were used in all experiments, but the list is presented to facilitate identification of cookie type when reference is made to a variety. It should be mentioned that these cookies are so-called sugar-snap cookies commonly baked by mill and bakery chemists for evaluating flours for soft wheat quality (2). Diameter data only are presented since there is a high correlation between diameter and spread factor (e.g.,  $r = 0.984$  for  $n = 14$ ),<sup>3</sup> and it is believed that the diameter value is more replicable than thickness, especially when only one or two cookies are measured.

In some instances, comparisons were made of the performance of a hard wheat flour with a good-quality soft wheat flour. The reason for this was that it was desired to have a large range in test results so that differences would be more obvious in these preliminary studies.

### Results

*Heating Mixograph.* Figure 1 presents the time vs. dough-consistency curves for heating-mixograph trials with several flours. The average temperature curve for the runs (which did not differ appreciably for the different samples) is shown as a dotted curve in the figure.

In Fig. 1 the time required for the various doughs to reach the stage of rapid increase in viscosity should especially be noticed. The Comanche (CO) dough was the first to show this increase, followed by Purkof (P), Trumbull (TR), Clarkan (CL), Blackhawk (B), and American Banner (A). This order is the approximate inverse varietal ranking of cookie diameter.

A further association between the time of viscosity increase and relative cookie quality is seen in Fig. 2. Two groups of curves are presented. On the left, the previous curves for Comanche and Clarkan have been repeated. On the right are curves for the same flours in which the dough samples were rolled, cut, and heated in the oven for varying periods, placed in the preheated bowl of the mixograph, and immediately mixed. In both cases the rapid viscosity increase came earlier for Comanche than for Clarkan. The oven-heated doughs were not, of course, subjected to mixograph mixing until each had been

<sup>3</sup> Unpublished data, Soft Wheat Quality Laboratory, Wooster, Ohio.

partially baked for a specified time. Thus, it may be said that these curves follow the viscosity changes that took place in cookie doughs as they occurred in the actual baking of cookies. On the other hand, the doughs heated in the mixograph (left, Fig. 2) had been mixed from the start of the application of heat. The temperature curves

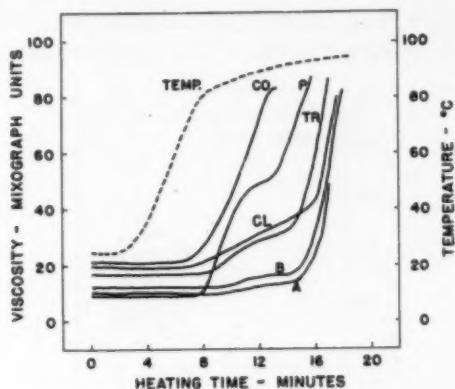


Fig. 1. Dough viscosity vs. heating time for cookie doughs mixed in the heating mixograph. Average temperature rise is indicated by dotted line. See Table 1 for variety legend.

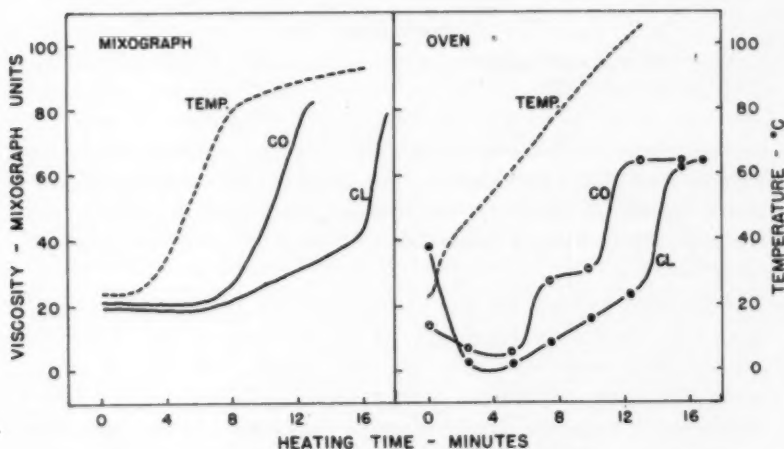


Fig. 2. Dough viscosity vs. heating time for Comanche (CO) and Clarkan (CL) cookie doughs heated in mixograph (left) and heated in oven followed by viscosity determination using the same instrument (right).

differ, especially at the higher levels. In spite of these differences, there appeared to be significant similarities in the two groups of curves.

*Heated Alkaline Water Retention Capacity.* The alkaline water retention capacity (AWRC) test is a measure of retention of a mildly alkaline solution by flour against centrifugal force. A highly significant negative correlation exists between retention results and cookie spread. The data obtained when AWRC determinations were made at several temperatures are presented in Fig. 3. The maximum temperature used

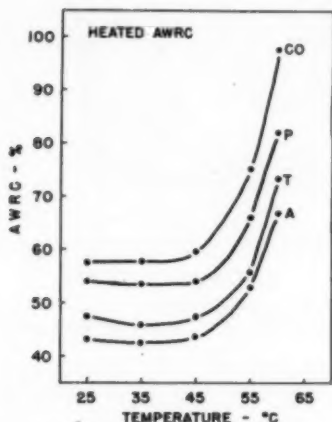


Fig. 3. Alkaline water retention capacity vs. temperature curves for flours. See Table I for variety legend.

in these experiments was 60°C. At higher temperatures, the gels formed were so voluminous and slack that replicate results could not be obtained. Retention values appeared to increase greatly at about 55°C. However, the rankings of flours remained the same in the temperature range covered.

*Viscograph Studies.* Because of the apparent importance of sugar as an ingredient in cookie doughs, it was incorporated into a mildly alkaline flour batter for viscograph tests. Figure 4 presents viscosity-temperature data with the use of flours from several varieties of wheat. As the figure indicates, large differences were found in the viscosities of these batters. Flours known to bake superior cookies formed slacker batters at the start of the test and required higher temperatures before showing a rapid increase in viscosity.

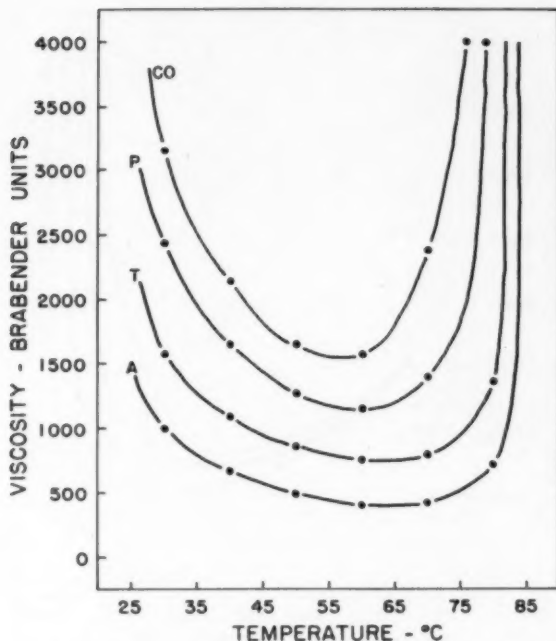


Fig. 4. Viscograph viscosity vs. temperature for flours using uniform water-flour ratio. See Table I for variety legend.

That limited changes in ratio of water to flour did not materially affect the picture is indicated in Fig. 5. Thus, for Comanche, the quantities of flour, water, and sugar were changed to 200 g., 243 ml., 120 g.; for Purkof, to 200 g., 228 ml., 120 g.; for Trumbull, maintained at 200 g., 200 ml., 120 g.; and for American Banner, changed to 221.3 g., 200 ml., 132.8 g., respectively. A gram of sodium bicarbonate was included in each test. With the use of these quantities of ingredients, an initial viscosity reading of 1580 Brabender Units was maintained while the flour-sugar ratio was kept uniform at 1 to 0.6. The temperature at which rapid viscosity increases occurred in these instances did not change materially, and doughs that contained flours considered to be superior for cookie purposes continued to require higher temperatures for viscosity increase. Moreover, lower viscosity levels were reached by the batters from better-quality varieties at the point of minimum viscosity. Since cookie dough absorptions are adjusted to optimum handling consistency, it appears that conditions illustrated in Fig. 5 would be more nearly comparable to actual baking than those in Fig. 4.

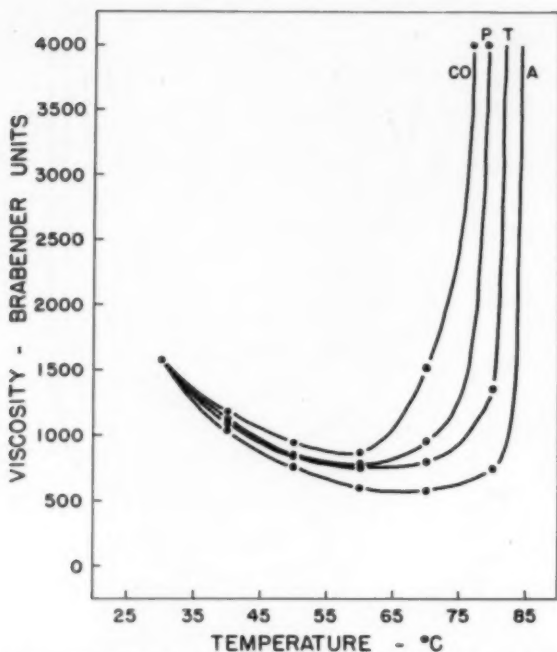


Fig. 5. Viscograph viscosity vs. temperature for flours using variable absorption to give identical initial viscosities. See Table I for variety legend.

*Studies of Baking Cookie Doughs.* Figures 6, 7, and 8 present the data on internal dough temperature vs. baking time, volatile matter loss vs. baking time, and cookie diameter vs. baking time, respectively, for cookie doughs baked for various periods in the oven.

It seemed that increase of internal dough temperature and loss rates of volatile matter did not differ materially for doughs of flours from different varieties. However, differences were noted in the spreading characteristics. For example, Comanche cookie doughs stopped spreading after baking for about 11 minutes, while those of American Banner continued to spread for 4 or 5 additional minutes, or a total of 15 to 16 minutes.

The relationship of baking time to rapid increase in viscosity for partially baked cookie doughs (right, Fig. 2) and to cessation of spread in cookies (e.g., Fig. 8) is illustrated in Fig. 9. In the figure, arrows indicate the point at which spreading stopped. These points for the doughs of the two flours coincide fairly well with the respective time intervals of rapid increase in viscosity.

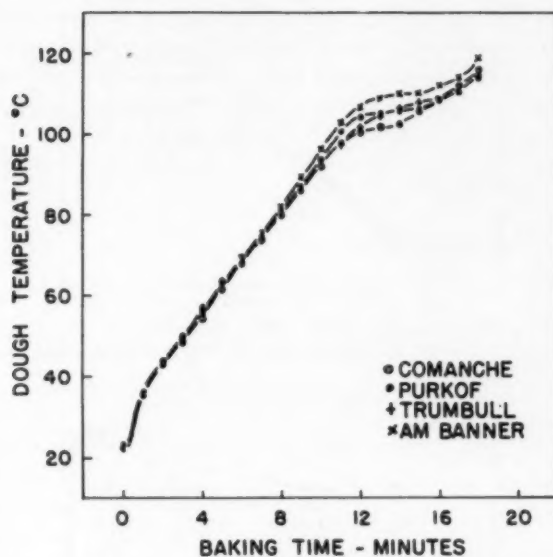


Fig. 6. Internal dough temperature vs. baking time for cookie doughs baked in the oven.

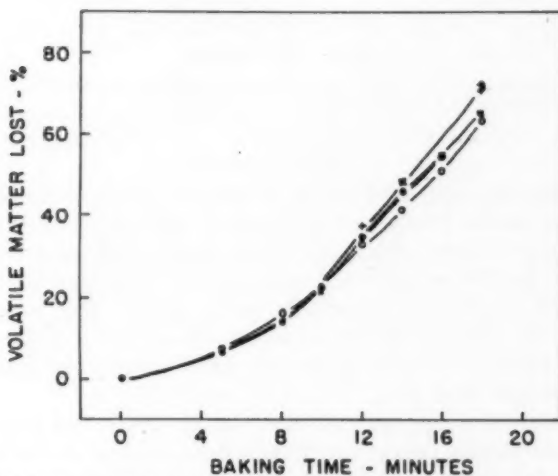


Fig. 7. Volatile matter loss vs. baking time for cookie doughs baked in the oven. For legend, see Fig. 6.

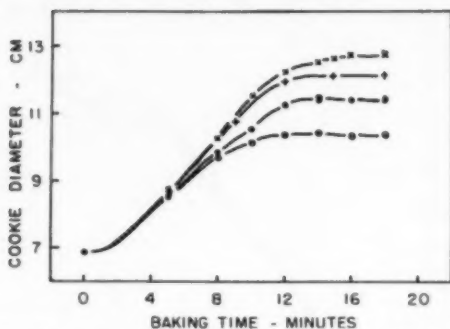


Fig. 8. Cookie diameter vs. baking time for cookie doughs baked in the oven. For legend, see Fig. 6.

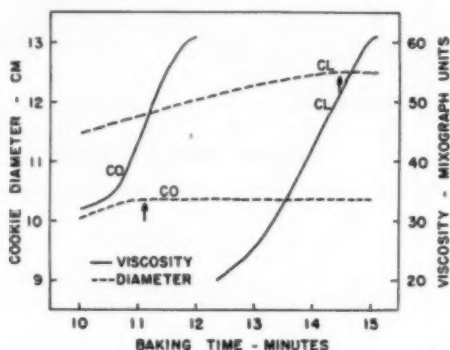


Fig. 9. Cookie diameter and dough viscosity vs. baking time for Comanche (CO) and Clarkan (CL) cookie doughs baked in the oven.

### Discussion

The data indicate that flours from different wheat varieties produce sugar-snap cookies which vary in spread in relation to the dough viscosity changes taking place. The coincidence of dough-setting time as determined by measurement of cookie diameter and the time of rapid viscosity increase of partially baked doughs (Fig. 9) is an example of this close association, as well as the similarity in the relative time for viscosity increases of partially baked doughs and mixograph-heated doughs (Fig. 2).

The heating-mixograph and oven-baking data reveal that doughs which make smaller cookies are those which also show an earlier rapid viscosity increase (and at a lower temperature) than those which make superior cookies. These results do not appear to reflect differences in

rates of heat penetration into the dough or in the rate of loss of volatile ingredients. Rather, changes in the status of the limited quantity of water present and intensive competition for the water among the various flour components, including gluten hydration, starch swelling and gelatinization, and the uptake of water by the starch tailings and solubles, may contribute materially to cookie quality.

#### Acknowledgment

The author is indebted to Lloyd Moser for technical assistance during the investigation.

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# THE BAKING QUALITY AND MALTOSE VALUE OF FLOUR IRRADIATED WITH $\text{Co}^{60}$ GAMMA RAYS<sup>1</sup>

C. C. LEE

## ABSTRACT

Loaf volumes of bread baked from two grades of flour (patent and baker's) were decreased by irradiation of the flours with  $\text{Co}^{60}$  gamma-rays at dosages of 0.25, 0.5, and 1.0 million roentgens. Recoveries of crude gluten from all the irradiated samples were also decreased. A marked adverse bromate effect, as well as an increase in the water-soluble nonprotein nitrogen, was found for the flours that were treated with  $10^6$  r. A regular increase in maltose value and gassing power with increasing dosages of radiation was observed for both grades of flour. However, no rise in saccharifying power was found for papain extracts of the irradiated flours, suggesting the absence of a possible activation of the beta-amylase in the flour. On the other hand, autolytic production of reducing sugars from mixtures of nonirradiated flour and irradiated starch was shown to increase with increasing irradiation of the starch. Similarly, rises in the saccharifying power were noted for papain extracts of nonirradiated flour when dispersions of irradiated starch were used as substrate. These findings strongly indicate that the increases in maltose value for the irradiated flours were due to an enhanced susceptibility of the starch in the irradiated flours to hydrolysis by beta-amylase.

A preliminary study of the effects of  $\text{Co}^{60}$  gamma-rays on the baking quality of cake, all-purpose, and bread flours was reported in 1955 by Brownell *et al.* (3). For the bread flour, some decrease in baking quality was noted, especially in treatments with the larger dosages of one-half and one million rep. More recently, Lloyd, Milner, and Finney (5) demonstrated that irradiation of wheat gluten, when dry or in aqueous suspension, with doses of X-rays up to 700,000 rep, resulted in decreases in viscosity of sols prepared from the irradiated gluten. These authors suggested that such results indicated that treatments of wheat or flour with ionizing radiation would have significant effects on the physical properties and behavior of gluten during dough mixing and fermentation processes. In this connection, Milner and Yen (7,8) have investigated the breadmaking and related properties of flour derived from a high-quality wheat which had been treated with various doses of gamma-rays. The present paper reports observations made on two grades of commercial bread flour which have been subjected to irradiation with  $\text{Co}^{60}$  gamma-rays.

## Materials and Methods

Two lots of flour, milled from Western Canadian hard red spring

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wheat and designated as patent and baker's grades,<sup>2</sup> were obtained in the spring of 1956 from the mill of the Saskatchewan Wheat Pool. These flours had not been subjected to improver treatments. Their crude protein and ash contents, on a 14% moisture basis, are listed below:

	Crude Protein (N $\times$ 5.7) %	Ash %
Patent	11.5	0.38
Baker's	12.6	0.51

Batches of about 600 g. of flour, in sealed aluminum containers, were irradiated with Co<sup>60</sup> gamma-rays by the Atomic Energy of Canada, Limited, Chalk River, Ontario, at dosage levels of 0.25, 0.50, and 1.0 million roentgens.<sup>3</sup> The source consisted of three capsules each holding approximately 70 curies of Co<sup>60</sup>. The capsules could be removed radially within a lead castle and a high intensity of radiation resulted when all three capsules were brought up close to the containers of the samples (2).

Baking tests on all samples were carried out with the following formula: 100 g. flour, 3 g. yeast, 5 g. sucrose, 1.75 g. sodium chloride, 3 g. shortening, 4 g. nonfat dry milk, 0.1 g. ammonium dihydrogen phosphate, 0.3 g. nondiastatic malt, and absorption to suit. For the samples that received 10<sup>6</sup> r. of radiation, the effect of 10 p.p.m. of potassium bromate was also determined.

Comparisons were made on the crude protein contents of aqueous and 50% alcoholic extracts of the control flours and the flours that were treated with 10<sup>6</sup> r. These were carried out by 1) extraction of 5 g. of flour with 50 ml. of distilled water; 2) addition of trichloroacetic acid (final TCA concentration 5%) to precipitate the water-soluble proteins from the aqueous extract, leaving a supernatant containing nonprotein nitrogen; and 3) extraction of the water-extracted flour with 50 ml. of 50% aqueous alcohol. All fractions as well as the final residue and the original flour were analyzed for nitrogen.

Determinations of crude gluten, maltose value, and gassing power were made on all samples according to procedures outlined in *Cereal Laboratory Methods* (1). The maltose value of a sample is a measure of the autolytic production of reducing sugars. The determination involved the autolytic digestion of the flour for one hour at 30°C. in acetate buffer of pH 4.6-4.8 without any measurement of reducing

<sup>2</sup> The patent grade contains the first 50% of the total flour. The baker's grade contains the next 38% of the total flour.

<sup>3</sup> One roentgen is taken to be equivalent to 0.975 rad (4) for this material, one rad being equal to 100 ergs per g. of material treated.

sugars originally present. Saccharifying powers of papain extracts of the irradiated and control flours were estimated according to a method used for malts and barleys (9). Two grams of flour were extracted for 20 hours with 40 ml. of 1% papain solution. The extract was filtered twice through No. 4 Whatman filter paper and the saccharifying powers of 1-ml. aliquots of the filtrate determined according to the ferricyanide method used for malt (1).

Samples of starch prepared from the two grades of flour used in these experiments and Merck's soluble starch<sup>4</sup> were also irradiated with Co<sup>60</sup> gamma-rays at dosages of 0.25, 0.50, and 1.0 million r. Maltose values were measured for mixtures of 5 g. of nonirradiated flour and 2 g. of irradiated starch. In addition, saccharifying powers of papain extracts of nonirradiated flour were measured with dispersions of irradiated starch as substrate.

### Results and Discussion

Loaf-volume data for bread baked from irradiated flours are given graphically in Fig. 1 and the effects of 10 p.p.m. potassium bromate are shown in Table I. These results indicate that baking quality declines on irradiation with Co<sup>60</sup> gamma-rays at the dosage levels em-

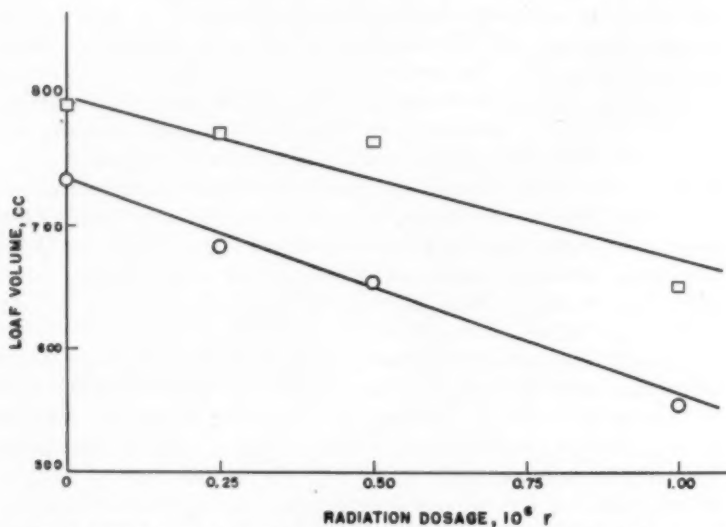


Fig. 1. Loaf volume of bread baked from irradiated flour. Open circle, patent flour; open square, baker's flour.

<sup>4</sup> Merck's soluble starch, according to Lintner, special for diastatic power determinations.

TABLE I  
LOAF VOLUMES OF BREAD BAKED WITH AND WITHOUT 10 P.P.M. POTASSIUM BROMATE

RADIATION DOSAGE	LOAF VOLUME*			
	PATENT FLOUR		BAKER'S FLOUR	
	Without Bromate	With Bromate	Without Bromate	With Bromate
r	cc	cc	cc	cc
0	740	750	800	825
10 <sup>6</sup>	555	470	650	600

\* Mean value of at least duplicate loaves. Deviations of individual values from the mean were not more than 29 cc.

ployed in these experiments. This is in agreement with the findings of Milner and Yen (8), who noted a decrease in crumb and loaf-volume characteristics for bread baked with flour milled from wheat that had been irradiated at dosages beyond 250,000 rep.<sup>5</sup> That changes in the flour proteins may at least be partly responsible for the deterioration in baking quality is indicated by the poor recoveries of crude gluten from the more highly irradiated samples of flour (see table below).

Dosage 10 <sup>6</sup> r.	Crude Gluten	
	Patent flour	Baker's flour
	%	%
0.0	12.2	13.8
0.25	12.1	13.4
0.50	11.5	13.2
1.0	7.1	10.7

TABLE II  
CRUDE PROTEIN CONTENTS (N  $\times$  5.7) OF FRACTIONS DERIVED FROM WATER AND 50% ALCOHOL EXTRACTS OF FLOUR

FLOUR	DOSAGE	PROTEIN CONTENTS					
		Unextracted Flour	Water- Soluble Protein	Water-Soluble Nonprotein Nitrogen (N $\times$ 5.7)	50% Alcohol- Soluble Protein	Residual Flour	Total Recovery
	r	%	%	%	%	%	%
Patent	0	11.5, 11.5	1.4, 1.3	0.4, 0.4	3.7, 3.8	5.6, 5.5	11.1, 11.0
	10 <sup>6</sup>	11.6, 11.7	1.4, 1.4	0.7, 0.7	3.4, 3.7	6.0, 5.6	11.5, 11.4
Baker's	0	12.6, 12.6	1.2, 1.0	0.4, 0.4	4.8, 4.5	6.4, 6.5	12.8, 12.4
	10 <sup>6</sup>	12.8, 12.7	0.9, 0.8	0.6, 0.6	4.6, 4.6	6.6, 6.5	12.7, 12.5

The results of crude protein analyses on the various fractions derived from the water and 50% alcohol extractions of the control flours and the flours treated with 10<sup>6</sup> r. are shown in Table II. For most of these fractions, there were little, if any, significant differences in nitro-

<sup>5</sup> At lower dosage levels, improvements in baking quality have been reported (6,8).

gen contents. However, the water-soluble nonprotein nitrogen fraction does appear to be greater than the control for both irradiated patent and baker's flours. Possibly, at the irradiation level of  $10^6$  r, some

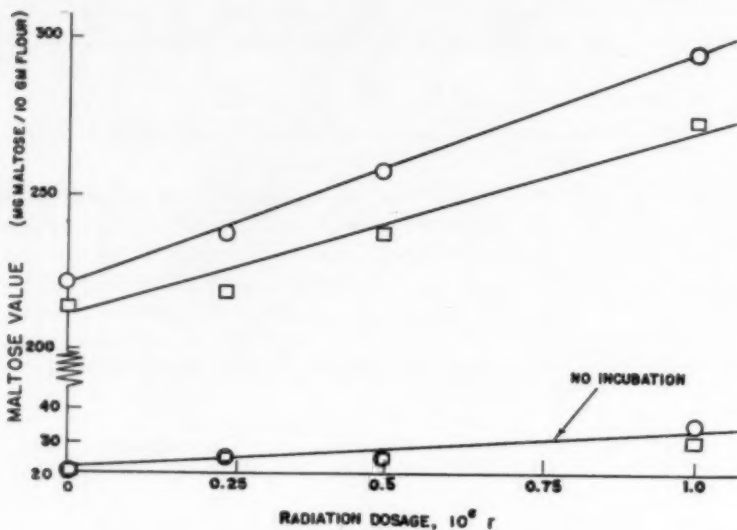


Fig. 2. Maltose value of irradiated flour. Open circle, patent flour; open square, baker's flour.

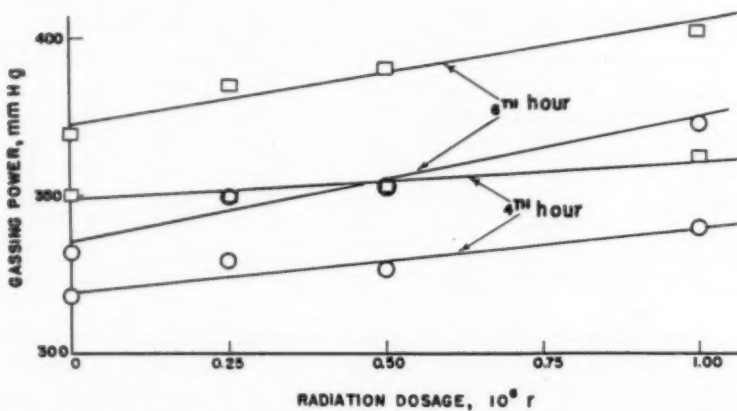


Fig. 3. Gassing power of irradiated flour. Open circle, patent flour; open square, baker's flour.

fragmentation of nitrogen-containing compounds might have occurred, giving rise to greater amounts of nonprotein nitrogen.

The maltose values of the irradiated flours, which, as pointed out before, are indicative of the amounts of reducing sugar produced after incubation of the samples for 1 hour at 30°C. and pH 4.6-4.8, were found to increase regularly with increasing dosages of radiation (Fig. 2). Corresponding rises in the gassing power of doughs prepared from the irradiated flours were also noted (Fig. 3). That this increased reducing sugar production must have arisen from some autolytic process was demonstrated by the fact that without incubation, the maltose values were very low for both control and irradiated flours (Fig. 2). Similar increases in maltose value with increasing radiation dosage were found by Milner and Yen (7,8) for flours derived from irradiated wheat. These workers pointed out the two possibilities that irradiation may either activate the beta-amylase or render the starch more susceptible to the enzymatic hydrolysis. The latter explanation was favored from considerations of the changes in viscosity of starch during gelatinization in the amylograph (7).

In an attempt to shed further light on the apparent increase in flour diastatic activity, as measured by maltose values, with increasing radiation dosage, the saccharifying power of papain extracts of the irradiated flours was determined. Experiments were also carried out with irradiated starch. For malts and barleys, digestion with papain is regarded as a means of extracting both free and bound beta-amylase, thus affording a measure of the total saccharifying power of the malt or barley (9). If the flour beta-amylase is activated by gamma-irradiation, papain extracts of irradiated flours would show an increase in saccharifying power. Actually, the saccharifying powers of the papain extracts of the irradiated flours were a little lower than those of the control flours (see table below), and thus no activation of beta-amylase by gamma-rays occurred. If, on the other hand, irradiation causes an enhanced susceptibility of the flour starch towards enzymatic hy-

Dosage 10 <sup>6</sup> r.	Saccharifying Power <sup>a</sup>	
	Patent flour	Baker's flour
	%L	%L
0.0	240	269
0.25	227	253
0.50	209	242
1.0	213	220

<sup>a</sup> Mean value of at least duplicate determinations. Deviations of individual values from the mean were not more than 2%L.

TABLE III  
AUTOLYTIC SUGAR PRODUCTION FROM MIXTURES OF  
5 G. OF FLOUR AND 2 G. OF IRRADIATED STARCH

MIXTURE	DOSE ON STARCH, $10^5$ R.	MALTOSE PER 10 G. FLOUR <sup>a</sup>
Patent flour alone		248
Patent flour and Merck's soluble starch	0.0 0.25 0.50 1.0	270 276 282 285
Patent flour and starch from patent flour	0.0 0.25 0.50 1.0	295 299 312 322
Baker's flour alone		218
Baker's flour and Merck's soluble starch	0.0 0.25 0.50 1.0	251 251 257 267
Baker's flour and starch from baker's flour	0.0 0.25 0.50 1.0	251 261 264 264

<sup>a</sup> Mean value of at least duplicate determinations. Deviations of individual values from the mean were not more than 3 mg. per 10 g. flour.

TABLE IV  
SACCHARIFYING POWER OF PAPAIN EXTRACTS OF FLOUR  
WITH IRRADIATED STARCH AS SUBSTRATE

MATERIALS	DOSE ON STARCH $10^5$ R.	SACCHARIFYING POWER <sup>a</sup>
Extracts of patent flour. Dispersions of Merck's soluble starch	0.0 0.25 0.50 1.0	246 250 254 256
Extracts of patent flour. Dispersions of starch from patent flour	0.0 0.25 0.50 1.0	222 231 237 244
Extracts of baker's flour. Dispersions of Merck's soluble starch	0.0 0.25 0.50 1.0	270 274 278 285
Extracts of baker's flour. Dispersions of starch from baker's flour	0.0 0.25 0.50 1.0	238 246 250 257

<sup>a</sup> Mean value of at least duplicate determinations. Deviations of individual values from the mean were not more than 2°L.

drolisis, the use of irradiated starch as substrate in the estimation of maltose value should result in an increased production of reducing sugars. This was actually found to be the case. Maltose values obtained from incubation of mixtures of 5 g. of nonirradiated flour and 2 g. of irradiated starch are shown in Table III. The saccharifying power of papain extracts of nonirradiated flour, measured with the use of dispersions of irradiated starch as substrate, are given in Table IV. Both of these determinations showed increases in apparent beta-amylase activity with increasing irradiation of the starch. The increased autolytic production of reducing sugars in irradiated flours is, therefore, very likely due to some modification of the flour starch by the gamma-rays, resulting in an enhanced susceptibility of the starch to hydrolytic cleavage by beta-amylase.

#### Acknowledgments

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## DISCOLORATION IN RICE: SOME STUDIES ON ITS NATURE AND EFFECT ON NUTRITIVE VALUE<sup>1</sup>

H. S. R. DESIKACHAR, S. K. MAJUMDER, S. V. PINGALE,  
AND V. SUBRAHMANYAN

### ABSTRACT

The brownish discoloration of a commercial sample of rice could not definitely be attributed to the Maillard reaction. The browning caused a slight reduction in the solubility of the protein and its growth-promoting value.

Discoloration could be induced in white polished rice within 48 hours by incubation of the rice containing 25% moisture. Evidence showed that the discoloration is microbiological in origin.

It was recently reported from Japan that some samples of imported rice which had undergone a yellowish type of discoloration were infected with *Penicillium citrinum* Thom and *Penicillium islandicum* Sopp, which are known to elaborate toxic metabolites (3). Samples of rice on the Indian market, especially those stored in ill-ventilated godowns, frequently exhibit a similar or allied type of discoloration, although the Indian samples are generally more brown than yellow. It was, therefore, of prime importance to investigate the harmfulness or otherwise of discolored rice found in India and also to study the nature and origin of discoloration in rice. The present communication deals with some chemical studies to elucidate the nature of the discoloration, and with nutritional studies to evaluate the effect of the discoloration on the nutritive value of rice. Preliminary observations to indicate that the discoloration is primarily microbiological in origin are also reported here.

### Materials and Methods

The commercial sample of browned rice used for the chemical and nutritional studies was obtained from a commercial warehouse. It had been milled from paddy which had been dried incompletely before storage or which had got wet in the godown itself. A normal sample in the same variety served as a control. The laboratory sample of browned rice used in the rat growth studies was prepared in the laboratory from white polished rice by incubating it for 24-72 hours at laboratory temperature ( $26^{\circ} \pm 2^{\circ}\text{C.}$ ) after raising its moisture content to 25%.

<sup>1</sup> Manuscript received July 9, 1957. Communication from the Divisions of Biochemistry and Nutrition, and Storage & Preservation, Central Food Technological Research Institute, Mysore, India.

**Chemical Studies.** The solubility of the brown coloring matter in the rice in different aqueous, alcoholic, or organic solvents was first determined. The denaturation of the rice protein as a result of the browning was studied by determining its solubility in 0.2% sodium hydroxide solution. Nitrogen in the clear alkaline extracts was determined by a micro-Kjeldahl method. Evidence for the intervention of a Maillard type of reaction in bringing about discoloration was sought for by studying 1) the *in vitro* digestibility of the protein by proteolytic enzymes, 2) the amount of free amino groups of lysine in the protein, and 3) the ferricyanide reducing property and the fluorescence of extracts from the rice samples.

Proteolytic digestion with pepsin, trypsin, and papain was followed by measuring the increase in nonprotein nitrogen in the enzymatic digests of the browned and control rice samples. One milliliter of the enzyme solution was added to a 5% emulsion of the rice flour adjusted to the appropriate pH (2.0, 7.8, and 4.6, respectively), and at the end of 5, 24, and 48 hours, 10-ml. aliquots were added to equal volumes of 10% trichloroacetic acid solution and filtered. Nitrogen in 5 ml. of the filtrate was determined by the micro-Kjeldahl method. Fluorescence and the extent of ferricyanide reduction in acid extracts of rice were measured by the procedures described by Cole *et al.* (1) and Hlynka *et al.* (5), respectively. The amount of free amino groups of lysine in the rice protein was measured by the method of Lieben and Loo (6) in the Van Slyke apparatus. The estimations were carried out on aqueous and alkaline extracts of rice and also on a suspension of rice flour which had been repeatedly extracted with 0.1N hydrochloric acid solution. A crude whole-protein preparation from rice obtained by hydrolyzing the starch with salivary amylase was also included in the study.

**Nutritional Studies.** A commercial browned rice sample and a normal sample of the same variety (as control) were first fed to two groups of six rats each, and the protein efficiency ratio of the rice protein (at a 5% level) was determined over a period of 6 weeks by the rat growth method of Osborne, Mendel, and Ferry (8). The nitrogen balance of the animals on the respective rice diets was also measured during a 1-week period.

In a subsequent experiment, polished rice which had been browned in the laboratory was fed to rats. Two types of diet were employed in this study. The first diet had the composition: 77.1% rice flour, 5.0% *Cajanus cajan*,<sup>2</sup> 5.0% groundnut oil, 0.9% milk powder, 2.0% salt, and

<sup>2</sup> Formerly called *Cajanus indicus*. It is a pulse (legume) normally consumed as part of the diet in India, especially in the South.

10.0% leafy and nonleafy vegetables; a composition similar to that of the rice diet consumed by the poor sections of rice eaters over a large part of South India. The second diet had the composition: 85.0% rice flour, 8.0% groundnut oil, 1.0% cod liver oil, 4.0% Osborne and Mendel salt mixture, and 2.0% vitaminized starch. It contained rice as the sole source of protein (5% level) and was nutritionally complete with respect to vitamins and minerals. The first experiment lasted for 10 weeks, and three groups of ten rats each were used for the study. The second feeding test lasted for 8 weeks and contained twelve rats per group.

*Inducing Discoloration in Polished Rice.* Microscopic examinations during the preparation of browned rice in the laboratory indicated that the browning may have been caused by the development of microflora on the rice during incubation. Hence, a number of samples of rice (both comparatively fresh and old) were incubated at different temperatures from 20° to 55°C. after their moisture content was raised to 25%, and the effects of sterilization and incubation under aerobic and anaerobic conditions on the development of brown discoloration in the rice were noted. Sterilized rice samples were also inoculated with a suspension from the browned rice. Rice samples were sterilized in the present study by autoclaving at 12.5 lb. for 30 minutes.

### Results and Discussion

*Chemical Studies.* The brown color associated with the commercial samples was not soluble in aqueous or organic solvents and was combined with the protein fraction of the rice. It could, however, be rendered soluble by digestion with a proteolytic enzyme (but not by salivary amylase). As browning could be induced in white polished rice in the laboratory, the possibility of the color being contributed by the pigments of the rice bran during storage of the paddy had to be excluded. As browning in stored milk powder (4) and in sick wheat (7) is caused by the formation of the Maillard complex, detailed studies were carried out to ascertain whether the browning that occurs in the rice is also due to the Maillard reaction. But, as can be seen from Tables I and II, there was no difference in the enzyme digestibility or in the amount of bound amino groups of lysine in the rice proteins from the brown and control samples of rice, which should have been the case if extensive Maillard browning had occurred. The fluorescence and the ferricyanide reducing property of extracts from browned rice were the same as for control samples. The fact that the protein in browned rice was less soluble and more denatured than that in the control sample (33% for browned and 45% for control) would indicate

that the first stage of Maillard browning had occurred. Although the whole rice grain may appear quite brown or yellow, the discoloration appears very mild when the rice is powdered. The visibly discolored rice may therefore represent a mild stage of Maillard browning. The possibility of the browning being partly of an oxidative type mediated by enzymes cannot, however, be ruled out.

TABLE I  
RELEASE OF NONPROTEIN NITROGEN BY PROTEOLYTIC ENZYMES  
(Comparative data expressed in terms of mg. nitrogen solubilized per g. of rice)

TIME	PAPAIN		TRYPSIN		PEPSIN	
	Browned	Control	Browned	Control	Browned	Control
hours						
5	2.0	1.7	3.2	3.2	5.3	5.2
24	2.4	2.0	6.3	6.4	7.1	7.1
48	2.7	2.4	7.6	7.8	7.7	7.5

TABLE II  
RELEASE OF AMINO NITROGEN FROM RICE PREPARATIONS IN THE  
VAN SLYKE APPARATUS  
(Comparative data in terms of ml. nitrogen released  
per g. of rice at S.T.P. after 30 minutes)

	AQUEOUS EXTRACT	ALKALINE EXTRACT	RESIDUE AFTER EXTRACTION WITH ACID	RESIDUE AFTER AMYLASE DIGESTION
Browned	0.05	1.33	0.59	0.81
Control	0.06	1.33	0.61	0.78

*Nutritional Studies.* Nutritional studies on the commercial samples of discolored rice indicated that the browning did not reduce the thiamine content of the rice (1.6 $\gamma$ /g in control and browned samples), although, as can be seen from Table III, the growth-promoting value of the protein was reduced as a result of the browning; however, the nitrogen balances of the animals in the two groups were not different. Apart from this reduction in protein value, no toxic or fatal effects were noticeable when the browned rice was fed to the rats.

TABLE III  
NUTRITIVE VALUE OF "BROWNED" RICE PROTEIN

	GROWTH		AVERAGE NITROGEN INTAKE AND BALANCE IN 5TH WEEK OF EXPERIMENT	
	Protein Intake	Increase in Weight per Gram of Protein Intake	Intake	Balance
	g	g	mg	mg
Browned	6.0	1.2	435.5	153.2
Control	6.1	1.6	454.9	150.0

\* Standard error of the means is  $\pm 0.11$  (5 Degrees of Freedom).

When similar studies were carried out with browned rice prepared in the laboratory, starting from raw polished rice, no deleterious effect of the browning could be demonstrated (Table IV). In fact, the rat feeding studies showed that controlled browning in the laboratory may increase the growth-promoting value of the rice. Although this may not have an application in human nutrition, the increase in the general growth-promoting capacity of the browned rice in these rat studies has to be noted, and its causes need to be looked into.

TABLE IV  
NUTRITIVE VALUE OF RICE BROWNED IN THE LABORATORY

	FOOD RICE DIET COMPOSITION		5% PROTEIN DIET COMPOSITION	
	Average Daily Food Intake	Average Weekly Increase in Weight <sup>a</sup>	Average Daily Food Intake	Average Weekly Increase in Weight <sup>b</sup>
	g	g	g	g
Browned (24 hours)	9.8	8.3		
Browned (72 hours)	10.0	9.7	6.6	6.2
Control	8.9	6.7	6.6	6.5

<sup>a</sup> Standard error of the means is  $\pm 0.42$  (16 Degrees of Freedom).

<sup>b</sup> Standard error of the means is  $\pm 0.35$  (10 Degrees of Freedom).

TABLE V  
EFFECT OF INCUBATION OF RICE CONTAINING 25% MOISTURE ON THE BROWNING OF RICE

SAMPLE WITH AGE OF STORAGE	INCUBATION TEMPERATURE (AEROBIC) OF UNSTERILE SAMPLES FOR 48 HOURS					INCUBATION OF SAMPLE AT 25°C. AFTER STERILIZATION <sup>a</sup>
	18-20	25	30	37	55	
	°C	°C	°C	°C	°C	
Bezwada commercial (12 months)	— <sup>b</sup>	+++ <sup>b</sup>	+++	Pink	—	—
Pachodi commercial (12 months)	—	++	+++	Pink	—	—
Bangarasanna commercial (11 months)	—	++	++	Pink	—	—
G.E.B. 24 (4 months)	—	—	—	—	—	—

<sup>a</sup> Heat sterilization imparted a slight yellow tinge to the rice but no brown color of the type observed with unsterile samples developed. When the sterilized samples were incubated after inoculation with a cell suspension from the browned rice, the typical brown discoloration developed in the rice.

<sup>b</sup> Plus sign indicates brown shade; minus sign indicates absence of browning. Anaerobic incubation or incubation in vacuum of the unsterile rice samples did not induce discoloration in the rice.

*Inducing Discoloration in Polished Rice.* Data on the effect of incubation of moist rice samples under different conditions (Table V) show that pronounced discoloration can be induced within 48 hours in polished rice by incubation of the moist samples at 25°C. and at 30°C. A pinkish discoloration was found at 37°C., while at a higher temperature like 55°C. or a lower temperature like 20°C., visible discoloration failed to appear within 48 hours of incubation. The fact that sterilization completely prevented the browning, coupled with the observation that incubation under anaerobic or evacuated conditions failed to in-

duce the discoloration, can be interpreted to mean that the discoloration induced in polished rice is primarily microbiological in origin under the conditions of the experiment. That microflora preponderate in rice incubated under warm and humid conditions has been observed earlier by Del Prado and Christensen (2), as well as by Teunisson (9). The development of a visible discoloration has not been reported by these authors. The causes for the differential response towards browning between old and comparatively fresh rice observed in the present experiments need to be further investigated. The type of microflora growing on the incubated rice samples and on commercial samples of browned rice should also be studied.

*Commercial Considerations.* Insofar as the complete harmlessness of browned rice of commerce is concerned, the Indian samples examined so far have not produced any fatal effects. Preliminary microbiological examination has not revealed the presence of the harmful species *Penicillium islandicum* Sopp or *P. citrinum* Thom on Indian samples as has been reported for Japanese samples. Careful microbiological screening is necessary before a discolored sample of rice can be passed for consumption. The observation that the browning is accompanied by microbial activity further emphasizes the need for caution in using discolored samples of rice. Maintenance of conditions inimical to the proliferation of microflora, such as thorough drying of the paddy before storage, should be a primary step in preventing discoloration during bulk storage of paddy or rice.

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# EFFECT OF DRYING ON THE PHYSICAL PROPERTIES AND CHEMICAL REACTIVITY OF CORN STARCH GRANULES<sup>1</sup>

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## ABSTRACT

Starch granules dried in air developed a "case-hardened" shell that slowed reagent penetration and reduced granule reactivity. This decrease in reactivity exceeds the compensating effect of increased area due to cavitation and, hence, reduced total granule reactivity.

Starches air-dried at 45°C. to different moisture levels and with different degrees of cavitation showed small differences in gelatinization temperatures or viscometric properties. However, as moisture content was lowered, these starches showed decreased reactivity to oxidation, sulfation, and acetylation, although the extent of cavitation was increased. In oxidation and sulfation reactions, lyophilized starch reacted much faster than air-dried starches.

Electron microscopic observations of sections of starch granules soaked in mercuric chloride showed more rapid salt penetration into lyophilized starch than into a commercial air-dried starch.

Work with the electron microscope has shown that the method and extent of drying starch controls the number of cavitated granules (5). Since practically all cavities open on the granule surface, it might be presumed that cavitated granules would differ from solid granules in pasting behavior or in chemical reactivity. The present work was undertaken to determine whether such differences exist.

## Materials and Methods

*Isolation of Starch.* Undried corn kernels of approximately 50–60% moisture were obtained from common yellow field corn. The corn kernels were ground in water in a Waring Blendor and filtered through a double layer of cheesecloth. After centrifugation of the filtrate, the precipitate was extracted with 0.06N sodium hydroxide solution for 2 hours to remove protein and then was neutralized with 0.1N hydrochloric acid solution.

*Drying and Preparation of Samples.* Some granules were dried at 45°C. in an air oven to the desired moisture content. Moisture was determined by drying the starch to constant weight in an air oven at 110°C. Other granules (approximately 50% moisture content) were lyophilized (final moisture content 8%). Still other undried starch granules were dehydrated by being passed serially through ethanol solutions of 10, 25, 50, 75, and 100% concentrations, with three changes

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of the last. Granules were allowed to remain 4 hours in each concentration. Ethanol was removed finally under vacuum over calcium chloride. This starch contained 11% materials volatile at 110°C.

Granules used for electron microscope studies were embedded in methacrylate and sectioned for electron microscopy as described by Whistler and Thornburg (6). All granule sections prepared for electron microscopy were leached for 30 seconds with toluene to remove the methacrylate embedding material. The electron microscope was the RCA EMU-2D. Percentage of sections with cavities was determined as previously described (5).

*Swelling Properties.* Viscosity curves were obtained using the Brabender Viscometer. Gelatinization temperatures were determined using the apparatus described by Pfahler, Kramer, and Whistler (3).

*Mercury Diffusion.* Five grams each (dry weight basis) of lyophilized and commercial (10% moisture) corn starches were hydrated for 24 hours in distilled water at room temperature (27°C.). The water was decanted from the starch and 100 ml. of a 1% mercuric chloride solution added. Granules were kept under constant agitation at 25°C. and 5-ml. aliquot portions removed after 1, 3, 5, 7, and 9 hours. These granules were filtered on a fritted glass disk, washed with and placed in dioxane, and prepared for electron microscopic observations as described above. Depth to which the mercuric chloride penetrated was determined by measuring the thickness of the dark ring which was caused by the mercury atoms' deflection of the electron beam. An average depth of penetration was obtained from measurements on 15 different granule sections. Such measurements were of good comparative value although they do not directly give radial penetration.

*Oxidation.* Air-dried, alcohol-dehydrated, and lyophilized starches were rehydrated in water for 24 hours at room temperature (27°C.) and oxidized with 105 ml. of 0.0161N sodium hypochlorite solution per g. of starch. Ethanol-dehydrated starch had a level of cavitation (41%) between that of the two air-dried samples. Two series of oxidations were measured; one in phosphate buffer at pH 7 and one in carbonate-bicarbonate buffer at pH 9. Unconsumed hypochlorite was measured by titration of a 1-ml. aliquot portion with thiosulfate solution.

*Sulfation.* Starches were sulfated by a modification of the method of Wurzburg, Rutenberg, and Ross (7). Separate 5-g. portions of air-dried and lyophilized starch were rehydrated in water for 24 hours at room temperature (27°C.) and then placed in 100 ml. of a saturated sodium sulfate solution containing 2 or 3 g. of sulfur trioxide-triethy-

mine complex (1) and 1 g. of sodium hydroxide. They were allowed to react at 25°C. under constant agitation for 24 hours. After neutralization with 2N acetic acid solution, the samples were dialyzed for 24 hours against distilled water.

Starches with low degrees of substitution were made by the above procedure, using less of the complex. Sulfated granules were soaked in 1% mercuric chloride solution for 36 hours, dialyzed, and prepared for electron microscopic examination.

*Sulfate Determination.* Ester sulfate was hydrolyzed by refluxing with 200 ml. of a 5% nitric acid solution for 16 hours. After the solution was filtered, the sulfate was precipitated with a 10% barium chloride solution and weighed as barium sulfate.

*Acetylation.* Five-gram samples (dry-weight basis) of cavitated, air-dried starch granules were added to a mixture of 50 ml. pyridine and 35 ml. acetic anhydride. Enough water was added so that the total water content of the reaction mixture would be the equivalent of that in all the starches if they were of 42% moisture content. The reaction mixture was constantly agitated at 25°C. Ten-milliliter aliquot portions were removed after 10, 20, 30, 45, and 75 hours. These aliquot portions were placed in 190 ml. of methanol, centrifuged, and the residue washed with three 50-ml. portions of methanol, and the final methanol was removed under vacuum over calcium chloride. The amount of acetylation of each sample was determined by the method of Murray, Staud, and Gray (2).

### Results and Discussion

Small differences were found in the viscometric properties of starches air-dried at 45°C. to 33, 21, and 8% moisture (containing 25, 35, and 50% sections with cracks, respectively), as illustrated in Fig. 1. The starches had approximately the same gelatinization range, but air-dried and commercially heat-dried starches gelatinized somewhat more slowly than less completely dried granules. The presence of cavities does not seem to improve those characteristics of the granules which control swelling properties.

The rate of peripheral penetration of mercuric chloride into lyophilized starch was greater than that into a commercial corn starch, as shown in Fig. 2; yet both starches possessed approximately the same number of sections with cracks—52% for lyophilized starch and 49% for commercial starch. From these data it may be concluded that air-drying of starch reduces the penetrability of the granule surface.

The rate and extent to which starches dried in various ways react with hypochlorite solution are shown in Fig. 3. Differences in the man-

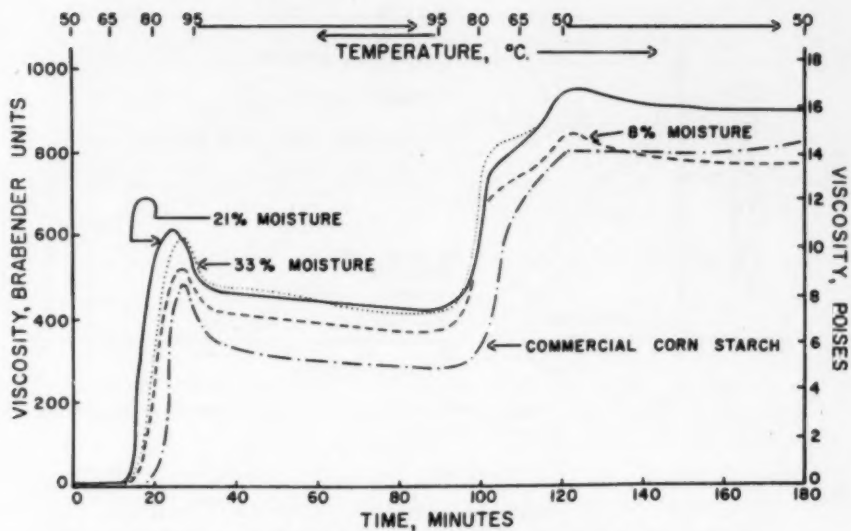


Fig. 1. Brabender viscosity curves of air-dried starches. Percent moisture given is the lowest to which the starch was dried. Concentration: 35 g. starch per 500 ml. of slurry.

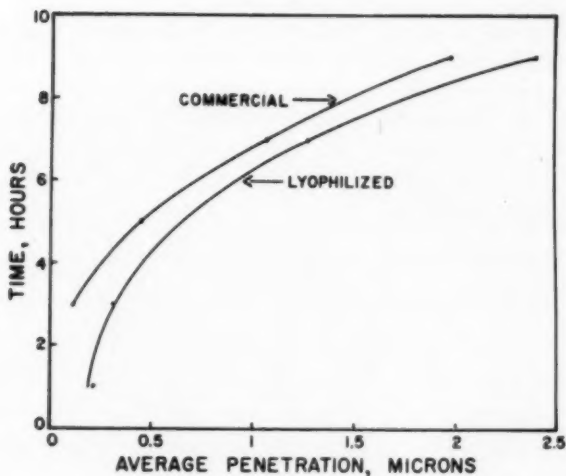


Fig. 2. Peripheral penetration of mercuric ions into starch granules.

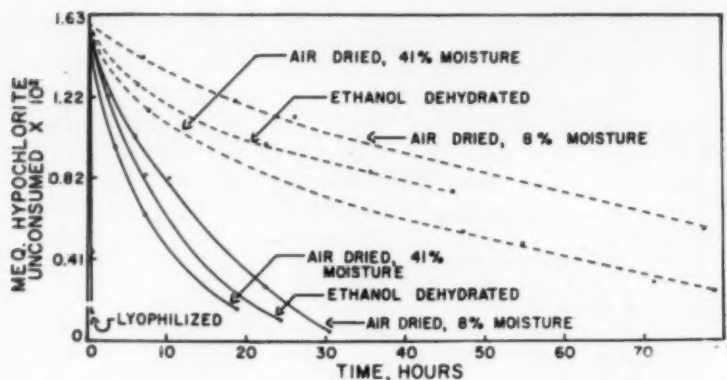


Fig. 3. Hypochlorite consumption by differently dried starches at pH 7 (continuous lines) and at pH 9 (discontinuous lines).

ner of drying, however, affect the rate of hypochlorite oxidation in inverse order to the degree of cavitation, except for lyophilized starch which is oxidized with remarkable rapidity. Oxidation is faster at pH 7 than at pH 9, in agreement with the results of Whistler and Schweiger for amylopectin (4).

A similar inverse order for rates of reactions was found in acetylations (Table I) and in sulfation (Table II), except that here again the lyophilized starch reacted most swiftly.

The decrease in chemical reactivity with decrease in moisture content in the oxidation, sulfation, and acetylation reactions may be due to physical changes in the surface of the air-dried granules, which surpass in their effect the increase in surface area brought about by cavitation. Partial rehydration of granules does not change the order in which the granules react to sulfation or oxidation.

TABLE I  
AMOUNT OF ACETYL GROUPS INTRODUCED INTO STARCHES DRIED TO DIFFERENT MOISTURE CONTENTS

TIME	ACETYL		
	42% Moisture (23% Sections with Cavities)	29% Moisture (27% Sections with Cavities)	9% Moisture (52% Sections with Cavities)
hours	%	%	%
10	13.8	12.2	7.9
20	15.8	14.3	10.9
30	17.5	15.6	12.2
45	17.6	15.9	13.2
75	19.0	17.1	13.8

TABLE II  
EXTENT TO WHICH VARIOUS STARCHES WERE SULFATED IN 24 HOURS AT 25°C.

DRYING CONDITIONS	SECTIONS WITH CAVITIES	MOLAR RATIO	
		Sulfating Complex: Anhydroglucose Units	
	%		%
Air-dried to 43% moisture	23	0.36	10.8
	23	0.54	12.0
Air-dried to 17% moisture	46	0.36	7.0
	46	0.54	8.6
Lyophilized	52	0.36	11.5
	52	0.54	12.9

Earlier work by Whistler, Spencer, Goatley, and Nikuni (5) showed that air-drying of starch sets up strains within the granules. Thus, it may be supposed that all starch granules dried in air, as well as those dried by certain other means, are physically stressed, and, in some, these stresses lead to partial or perhaps total release through cavitation. Such a concept of stressed granules may lead to a better understanding of granule action. Now, in addition to this physical state of stress, another condition must be added, namely, that of a differentiated shell. In the process of drying in air, and under certain other conditions, a surface layer of starch molecules, through kinetic motion, seems to become more tightly bonded intermolecularly. The resulting increase in degrees of association gives the molecules a "case-hardened" effect which influences the rate of diffusion of penetrating chemicals

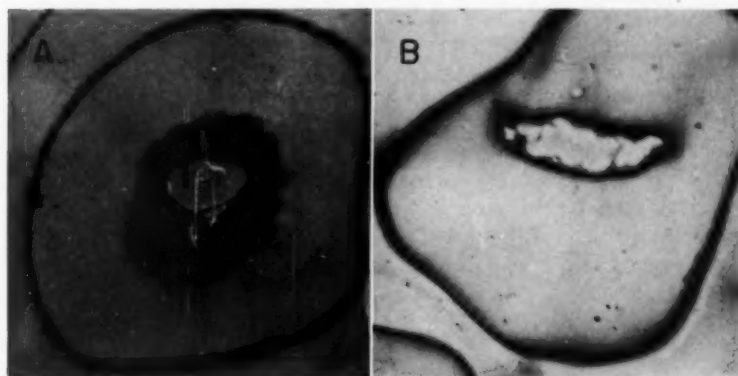


Fig. 4. Sections of sulfated starch treated with mercuric chloride: A, circular, highly reacted cavity; B, irregular, less reacted cavity.

and, consequently, the rate of chemical reactivity. The old concept that starch granules have a differentiated skin or shell should be revitalized with the modification that the shell is not chemically different but only physically different from the starch lying deeper in the granule.

Electron microscopic observations of sections of mercury-diffused sulfated starch suggested that two types of cavities were present. One type of cavity was nearly circular, as seen in granule sections illustrated by Fig. 4, A, in which the granule appears to have been sulfated to a greater depth from the cavity surface than from the external surface. The second type of cavity was irregular and usually contained sharp points. Here the granules appear to be sulfated to the same depth from the cavity as from the external surface (Fig. 4, B).

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## GELATINIZATION AND PASTING CHARACTERISTICS OF RICE VARIETIES AS RELATED TO COOKING BEHAVIOR<sup>1</sup>

JOHN V. HALICK<sup>2</sup> AND VINCENT J. KELLY<sup>3</sup>

### ABSTRACT

Amylograms of short- and medium-grain rice varieties generally exhibited lower gelatinization temperatures and shorter gelatinization times than did those of the long-grain varieties. Century Patna 231 (long-grain) and Early Prolific (medium-grain) with very high gelatinization temperatures were atypical, as was Toro (long-grain) with a low gelatinization temperature. While gelatinization temperatures were independent of amylose content, maximum viscosity of hot paste and gel formation on cooling were related to amylose content. Water uptake by whole rice at temperatures ranging from 72° to 82°C. was closely related to gelatinization characteristics in all varieties. The short- and medium-grain varieties, with low gelatinization temperatures (64.5°-67.5°C.), absorbed more water than did the long-grain types. Toro, the long-grain variety with a low gelatinization temperature (67.5°C.), was similar to the short- and medium-grain types in water-uptake behavior. The two varieties with high gelatinization temperatures, Early Prolific and Century Patna 231 (75.5° and 79.5°C., respectively), absorbed less water than any of the other varieties tested.

Rice varieties vary greatly in cooking and processing behavior. Since starch comprises approximately 90% of the dry matter of the rice endosperm, research on rice cooking behavior in recent years has largely centered on this fraction. Rao *et al.* (7) reported a relationship between amylose content and consumer acceptance of rice in India. More recently, Williams *et al.* (10) found that rice varieties with a high amylose content are fluffy and nonsticky when cooked. Halick and Keneaster (3), using an empirical method, also reported a relatively high amylose content to be associated with certain preferred cooking and processing characteristics in long-grain rice.

Measurements of water absorption by rice during boiling have been used to account for differences in cooking behavior for many years. Halick and Keneaster (3) found that the "swelling numbers" of Rao *et al.* (7) were not adequate to evaluate American rice varieties. The swelling number was described by these workers as the weight of the water imbibed by 100 g. of rice when cooked in water at 98°C. under standard conditions. Although differences were noted between the short-, medium-, and long-grain types, no differences were found between long-grain varieties known to vary greatly in cooking

<sup>1</sup> Manuscript received October 7, 1957. Contribution from the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, the Texas Agricultural Experiment Station, the Texas Rice Improvement Association, and Gerber Products Co.

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<sup>3</sup> Chemist, Research Department, Gerber Products Co., Oakland, California.

behavior. More recently, Hogan and Planck (4) reported that at 70°C. water-absorption values correlated with cooking and processing characteristics and with grain type. This correlation was less striking, especially between various long-grain varieties, when the determinations were made at 98°C.

To obtain more information on the influence of the starch fraction of rice on its cooking behavior, a study was made of the gelatinization and pasting characteristics. The results of these studies using a Brabender Amylograph in addition to water uptake at several temperatures are reported in this paper.

### Materials and Methods

The samples tested consisted of 17 rice varieties from the 1956 varietal test at Beaumont, Texas, together with one commercial sample of glutinous rice. Rice flour was ground in a "Homoloid" mill (W. J. Fitzpatrick Co., 1003 West Washington Blvd., Chicago, Ill.) equipped with a 3/16-in. screen. The flour was then sifted through a 40-mesh Tyler screen to remove any large particles, since it has been our experience that particle size greatly influences the pasting characteristics of rice. The Standard Model Amylograph (Brabender Corp., Rochelle Park, N. J.) was used in these studies. For the complete curve, a slurry was prepared by mixing 50 g. of rice flour and 450 ml. of distilled water in a Waring Blendor for 1.5 minutes. The slurry was then placed in the amylograph cup and heated to 30°C. At this point the chart was adjusted to a zero-minute marking, and the slurry was heated to a temperature of 94°C. at a rate of 1.5°C. per minute. The starch paste was maintained at this temperature for 20 minutes and then cooled at the rate of 1.5°C. per minute. The setback on cooling was evaluated at 50°C. The peak viscosity of the slurry of some varieties was so high that the recording pen went off the chart, and the peak viscosity had to be estimated.

Swelling of starch granules begins well before the effect of increased viscosity becomes apparent. According to Schoch (8), corn starch granules lose their birefringence in the range of 65° to 70°C.; but the viscosity does not begin to rise until 80°C., when the granules are swollen to approximately four times their original diameter. In these studies, at the concentration described above, the initial viscosity increase of the rice pastes was ill-defined and covered a range of several degrees. Therefore, to accentuate the gelatinization temperature, a separate determination was made on a slurry of 100 g. of ground rice and 400 ml. of water. The gelatinization temperature was taken as the point of initial increase in viscosity.

Most investigators agree that the measurements with this instrument are accurate to  $\pm 20$  Brabender units or less. With three replicate determinations on the same lot of rice, it was found that the gelatinization temperature could be estimated within the range of  $\pm 0.5^\circ\text{C}$ .

Starch-iodine blue values, which are inversely related to amylose content, were determined according to the method of Halick and Keneaster (3). The value of this method as a crude estimate of the relative amylose contents of rice was discussed by Williams *et al.* (10).

Whole kernels of fully milled and polished rice from the same lots described above were used in the water-uptake studies. Superficial fat and loose particles of bran and polish were removed by putting the rice in petroleum ether for 15 minutes, stirring at frequent intervals. The ether was then decanted and the rice spread on absorbent paper to dry. A measured amount of distilled water was pipetted into a large (25 by 100-mm.) test tube containing 2 g. ( $\pm 0.01$  g.) of the prepared rice. The amount of water added was based on an assumed excess of 6 to 8 ml., as determined by previous experiments. The tubes were stoppered lightly to minimize evaporation losses, allowed to stand for 30 minutes, and then immersed in the water bath for 45 minutes at the desired temperature. At the end of this time, the tubes were immersed in cold water immediately to stop the cooking process. The contents were then filtered through a Gooch crucible (size 3) with light suction into a graduated sedimentation tube, and the filtrate centrifuged at 1800 r.p.m. (head radius, 8.5 in.) for exactly 5 minutes. The sedimentation volume (representing insoluble solids) and the volume of unabsorbed water were read directly; whereas the absorbed water was determined by difference, taking into account the sedimentation volume. For convenience, the water uptake is reported as a "water-uptake number" which is arbitrarily calculated as the number of ml. of water absorbed by 100 g. of rice.

### Results and Discussion

The gelatinization and pasting characteristics, along with starch-iodine blue values of 18 rice varieties, are presented in Table I. It is clearly recognized that particle size, concentration, and other factors influence the size and shape of the amylograph curve. By holding these factors constant, however, the differences observed can be attributed to inherent varietal responses. In general, amylograph curves shown by the rice varieties were typical of those of other cereal starches, but they showed appreciable differences between varieties.

*Gelatinization Temperature and Time.* In general, the long-grain

varieties gelatinized at higher temperatures than the short- and medium-grain types, but there were some notable exceptions. Toro, a long-grain variety, had a gelatinization temperature (67.5°C.) similar to that of the short- and medium-grain types. This is in agreement with the reported "moist" appearance of this variety when cooked (10), a characteristic usually associated with medium-grain rice. Century Patna 231 had a gelatinization temperature of 79.5°C., which is much higher than any other of the varieties tested. This may partly explain the common observation that this variety has a compara-

TABLE I  
GELATINIZATION AND PASTING CHARACTERISTICS OF RICE VARIETIES

VARIETY	GELATIN- IZATION TEMPER- ATURE	GELAT- INIZA- TION TIME	TEMPERA- TURE AT PEAK VISCOSITY	VISCOSITY AT 94°C.				IODINE TRANS- MISSION
				Peak	Initial	After 20 Minutes	Cooled to 50°C.	
	°C	minutes	°C	BU	BU	BU	BU	%
SHORT-GRAIN								
Caloro	67.5	15	90.0	1100	850	440	760	64
Colusa	66.0	17	91.5	1050	980	430	790	48
"Glutinous"	58.0	6	67.5	290	120	110	170	Red
MEDIUM-GRAIN								
Early Prolific	75.5	7	87.5	1200	910	460	800	86
Calrose	67.5	15	90.0	1100	900	470	800	77
Magnolia	66.5	16	90.0	1050	760	420	750	70
Blue Rose	64.5	18	91.5	900	700	400	710	67
Nato	67.5	17	92.5	1050	1000	440	760	65
Zenith	65.0	18	91.5	1050	820	470	820	54
LONG-GRAIN								
Century Patna 231	79.5	7	90.0	1100	790	480	820	88
Toro	67.5	16	91.5	1050	870	440	750	75
Sunbonnet	72.5	12	91.5	1000	870	450	870	35
Bluebonnet 50	72.0	13	91.5	920	700	390	760	35
Fortuna	70.5	14	91.5	1000	800	420	800	34
Imp. Bluebonnet	72.5	12	91.5	920	850	460	920	31
Rexoro	73.5	11	91.5	820	790	450	930	26
TP 49	73.5	12	92.0	820	800	430	900	25
Texas Patna	70.5	14	91.5	800	700	440	900	21

tively long cooking time. This characteristic is not exhibited by the parental varieties, Texas Patna, Rexoro, and Blue Rose, from which Century Patna 231 was developed. The undesirable cooking and processing characteristics of Century Patna 231 have been reported previously (3). Early Prolific, a medium-grain variety, also exhibited a high gelatinization temperature (75.5°C.). While this variety is no longer commercially important, it also was noted for its undesirable cooking quality and long cooking time (9).

The low gelatinization temperatures of the short- and medium-grain varieties should be of interest to brewers and cereal manufacturers using diastatic digestion in their processes. A low gelatinization

temperature allows complete liquefaction of starch before thermal inactivation of the enzyme can occur.

Gelatinization temperatures of six lots of the Zenith variety, obtained from six locations in Louisiana, Arkansas, and Mississippi, ranged from 62.2° to 69.7°C. with a mean of 65.0°C. This indicates that environmental conditions also influence the pasting characteristics of rice varieties.

The gelatinization time was taken as the number of minutes required to reach peak viscosity, starting from the first perceptible increase in viscosity. Very short gelatinization times were exhibited by the "glutinous," Early Prolific, and Century Patna 231 varieties. The latter two varieties recorded not only the highest gelatinization temperatures (75.5° and 79.5°C.) of all varieties tested, but also the lowest temperatures (87.5° and 90.0°C., respectively) at peak viscosity. The low gelatinization and peak viscosity temperatures (58.0° and 67.5°C., respectively) of the "glutinous" variety with its subsequent short gelatinization time may be an indication of a homogeneous starch composition. Except for the three varieties mentioned above, gelatinization times were associated with grain type. Gelatinization times for the long-grain varieties ranged from 11 to 14 minutes, while those of the short- and medium-grain types ranged from 15 to 18 minutes.

*Viscosity.* Generally, varieties with the highest amylose contents showed the lowest peak viscosities. This is evidenced by the close relationship between the iodine transmission values and the peak viscosities, except in the Blue Rose and "glutinous" samples.

The rice pastes were typical of granular starches, in that they thinned during the 20-minute cooking period. It has been reported (5) that the increase in viscosity on cooling to 50°C. reflects the retrogradation tendency of the starch product. It then follows that high-amylose starches would show maximal setback on cooling. When cooled to 50°C., Texas Patna, Rexoro, and TP 49 showed maximum increases in viscosity, even exceeding the peak viscosities recorded by the hot pastes.

It becomes increasingly apparent that the nonamylose fraction, "amylopectin," of rice starch may be more influential in cooking behavior than previously thought. Apparently qualitative as well as quantitative differences are present. For example, Century Patna 231 and Toro have approximately the same amylose content (10), yet the gelatinization temperatures of these varieties are widely different. Thus, the gelatinization characteristics of rice starches do not depend entirely on their amylose content. A particular case in point is illus-

trated by the pasting behavior of the glutinous or "waxy" rice. According to Meyer and Fuld (6), glutinous rice starch is composed entirely of highly branched molecules of low and medium molecular weight. The gelatinization and pasting characteristics of glutinous rice, in particular the low temperature and viscosity, are indicative of a starch of low molecular weight. The nonamylose or "amylopectin" fractions of the common rice varieties, on the other hand, have molecular weights of an apparently higher order. It is suggested that amylopectin of waxy rice differs from that of waxy sorghum or maize. These latter two starches show very high hot-paste viscosities when compared to ordinary corn or sorghum (2,5). While the results reported herein were based on a commercial sample of waxy rice of unknown origin, comparable results have been obtained on two waxy varieties from the Beaumont breeding nursery.

*Water Uptake.* Water-uptake numbers of 17 varieties at three temperatures are shown in Table II. It is immediately apparent that water uptake is closely related to the gelatinization characteristics as determined by the amylograph. This is not surprising, since viscosity is a function of water imbibition followed by (or possibly concurrent with) swelling of the starch granules. The short- and medium-grain varieties, with low gelatinization temperatures ( $64.5^{\circ}$  to  $67.5^{\circ}\text{C.}$ ), ab-

TABLE II  
WATER-UP TAKE NUMBERS OF RICE VARIETIES

VARIETY	WATER-UP TAKE NUMBER		
	72°C.	77°C.	82°C.
SHORT-GRAIN			
Caloro	249	432	502
Colusa	227	412	462
MEDIUM-GRAIN			
Early Prolific	60	88	177
Calrose	135	327	439
Magnolia	108	248	352
Blue Rose	132	303	417
Nato	105	303	422
Zenith	110	321	456
LONG-GRAIN			
Century Patna 231	54	78	147
Toro	121	371	499
Sunbonnet	52	93	320
Bluebonnet 50	60	90	316
Fortuna	80	148	363
Improved Bluebonnet	59	99	308
Rexoro	58	102	331
TP 49	59	108	361
Texas Patna	62	129	395
L.S.D. 0.05	13	15	16
0.01	17	21	21

sorbed more water at the lower temperatures than did the long-grain types. Toro, the long-grain variety with a low gelatinization temperature (67.5°C.), was similar to the short- and medium-grain types in water-uptake behavior. The two varieties with high gelatinization temperatures, Early Prolific and Century Patna 231 (75.5° and 79.5°C., respectively), absorbed less water than any of the other varieties tested.

These observations are at complete variance with results obtained at higher temperatures. Batcher *et al.* (1) reported that, at approximately 98°C., the long-grain types absorbed more water than did the short- and medium-grain varieties. It is suggested that at the higher temperatures water uptake may become a function of size and shape of the endosperm, and varietal differences in composition are then masked.

Sedimentation volumes increased sharply as the range of initial swelling was approached. At the present time the significance of the sedimentation volumes is obscure.

Practical aspects of these observations are felt to be of great value. Where time or facilities prohibit or limit running the complete Brabender curve, a rough estimate of the gelatinization characteristics of rice varieties can be made by determining water uptake at a series of temperatures. In screening hybrid material at the Rice Quality Laboratory at Beaumont, Texas, the determinations are made at 77° and 82°C. At the lower temperature it is possible to distinguish the typical short- and medium-grain reaction; while at 82°C., varieties gelatinizing at high temperatures, such as Early Prolific and Century Patna 231, are readily identified.

#### Acknowledgments

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## A NOTE ON EXPERIMENTAL WET-MILLING OF HIGH-AMYLOSE CORN<sup>1</sup>

R. A. ANDERSON AND V. F. PFEIFER

The Northern Utilization Research and Development Division has been cooperating for several years with a commercial corn breeder and with corn breeders in Production Research, Agricultural Research Service, U. S. Department of Agriculture, stationed at the Missouri Agricultural Experiment Station. These workers are attempting to develop field types of corn with starch containing 80 to 100% amylose (4,5). The breeders are also trying to maintain the starch content in this corn within the range of common field corn. To date, samples of corn have been found with starch having an amylose content up to 82%, as compared to the 27% present in ordinary corn starch, but only very small samples of these have been available.

This note reports results obtained in a series of wet-milling experiments carried out on a sample of high-amylose dent-type corn<sup>2</sup> which was made available in sufficient quantity for preliminary processing studies and to produce enough high-amylose starch for research purposes. This corn contained 63.6% starch, which had an amylose content of 54.5%. Experiments were carried out on both a bench scale and in a 4-bu. batch pilot plant.

Past experience in processing small samples of high-amylose corn indicated that considerable difficulty would be encountered in separating starch from gluten because of the small size of the starch granules (4). In some previous experiments at this laboratory, the starch recovered from high-amylose corn contained about 1% protein, as compared

<sup>1</sup> Manuscript received March 10, 1958. Contribution from the Northern Utilization Research and Development Division, Peoria, Illinois, one of the Divisions of the Agricultural Research Service, U. S. Department of Agriculture.

<sup>2</sup> Corn for these studies was made available through the courtesy of the American Maize-Products Co. and National Starch Products, Inc. The sample was a composite of yields from several strains developed by the Bear Hybrid Corn Co.

to 0.3–0.5% in starch usually recovered by wet-milling common dent corn.

Prior to carrying out the pilot-plant processing, a number of laboratory experiments were conducted on 1,500-g. lots of corn to determine suitable conditions for maximum recovery of good-quality starch. Batches of 200 lb. were then processed in the pilot plant.

Each 1,500-g. batch of corn was steeped in distilled water containing sulfur dioxide. At the completion of steeping, the water was drained, and the grain was ground twice in a Quaker City<sup>3</sup> drug mill. To remove coarse fibers, the resulting slurry was screened on a 0.039-in. perforated copper sieve. It was then rescreened on a 200-mesh stainless-steel sieve to remove fine fibers. Each fiber fraction was washed to remove starch. The final slurry, the mill starch, was tabled to separate the starch from the gluten. This procedure has been described in detail by Zipf *et al.* (8).

In the pilot-plant runs, 200 lb. of grain were steeped in tapwater containing sulfur dioxide. The water was drained, and the grain was ground twice—first through a Bauer mill equipped with jaw-tooth plates and then through a Rietz disintegrator equipped with a 1/16-in. round, perforated screen. Coarse fibers were removed from the slurry by passing it over a Rotex gyratory shaker equipped with a 26-mesh screen, and fine fibers were recovered by passing the slurry over the same shaker fitted with a 200-mesh screen. The resulting mill starch was separated into starch and gluten by tabling. The pilot-plant equipment and procedures have been described by Anderson (2).

Protein in the starch ( $N \times 6.25$ ) was determined by the Kjeldahl-Gunning-Arnold method (1). Starch content of the corn was determined polarimetrically by the procedure of Earle and Milner (6). The amylose content of the separated starch was determined by the iodine sorption method of Bates, French, and Rundle (3), as modified by Wilson, Schoch, and Hudson (7). Moisture in corn or starch was determined by drying samples for 4 hours at 110°C. under a vacuum of 28 in. of mercury. Sulfur dioxide in the steepwater was determined by titration with iodine solution using starch as the indicator.

Pertinent processing and analytical data are given in Table I. When high-amylose corn was processed under standard operating procedure (run 1), no separation of starch and gluten was obtained by tabling, but separation was obtained when the mill starch was centrifuged in cups in an International No. 2 centrifuge. Such starch had a protein content of 0.69% on a moisture-free basis. Decreasing the pitch of the table from 1.0 in. per 20 ft. to 0.5 in. (run 2) permitted

<sup>3</sup> Mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

TABLE I  
PROCESSING AND ANALYTICAL DATA FROM THE WET-MILLING OF HIGH AMYLOSE CORN

UNIT	RUN	LABORATORY					7-9 <sup>d</sup>
		1 <sup>a</sup>	2	3	4	5 <sup>b</sup>	PILOT PLANT
Steeping data:							
Initial SO <sub>2</sub> content, % by wt.		0.24	0.26	0.25	0.26	0.25 <sup>b</sup>	0.25
Initial pH		1.90	1.85	2.00	2.10	2.30	1.95
Time, hours at 125°F.		66	48	48	48	50	48
Tabling data:							
Specific gravity, °Be at 60°F.		6.0	6.0	3.4	3.2	3.5	3.5
pH		4.4	4.4	4.6	.....	4.0	5.1
Table length, ft.		20	20	20	20	20	46
Table width, in.		3.4	3.4	3.4	3.4	3.4	12
Pitch of table, %		0.42	0.21	0.21	0.21	0.21	0.45
Rate of flow, gal/minute		0.079	0.079	0.079	0.079	0.079	0.75
Analytical and recovery data:							
Starch recovery, % M.F.B. <sup>e</sup>	*		82.6	76.9	82.5	84.8	79.2
Protein in starch, % M.F.B.	0.69		1.00	0.59	0.66	0.73	0.81
							0.72

\* Starch could not be recovered by tabling. It was centrifuged in cups, and the gluten was scraped from the starch layer.

<sup>b</sup> After 48 hours of steeping, SO<sub>2</sub> content was increased to 0.55% and steeping continued for 2 hours.

<sup>c</sup> After 48 hours of steeping, SO<sub>2</sub> content was increased to 0.38% and steeping continued for 2 hours.

<sup>d</sup> Average of three runs.

\* Moisture-free basis.

some separation of starch and gluten, but the protein content of the starch was still 1.0%.

Reducing the specific gravity of the mill starch from the usual 6° Bé at 60°F. to 3.2°–3.5° resulted in the best separation (runs 3 and 4). However, the starch did not settle to a hard cake, and it was necessary to wash the gluten carefully from the surface of the starch.

Additional sulfur dioxide was added after 48 hours of steeping in an attempt to improve starch-gluten separation (runs 5 and 6), and steeping was continued for another 2 hours; no improvement was noted in ultimate separation, although starch was more readily washed from the fibers and starch recovery was slightly greater.

Three pilot-plant experiments were made for the purpose of preparing a 300-lb. sample of starch. Processing conditions established in the laboratory tests were used, and observations made were similar to those noted in the small-scale work. The recovered starch had a protein content of 0.72%, moisture-free basis.

The texture of the high-amylose starch was soft and thin, and when examined microscopically the granules were spherical and smaller in size than those of ordinary corn starch.

This brief investigation was conducted using a starch table for starch-gluten separation. Similar or better separation would be expected with a commercial centrifuge of the type used in the starch industry.

Processing studies on high-amylose corn will be continued as new samples are provided by corn breeders. There is a possibility that greater difficulty in processing will be encountered as the amylose content of the starch is increased.

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**General.** Authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* 6:1-22, 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

**Editorial Style.** A.A.C.C. publications are edited in accordance with *A Manual of Style*, University of Chicago Press, and *Webster's Dictionary*. A few points which authors often treat wrongly are listed below:

Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°-10°C.). Place 0 before the decimal point for correlation coefficients ( $r=0.95$ ). Use \* to mark statistics that exceed the 5% level and \*\* for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g.,  $A/(B+C)$ . Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the *Style Manual* or the *Dictionary*.

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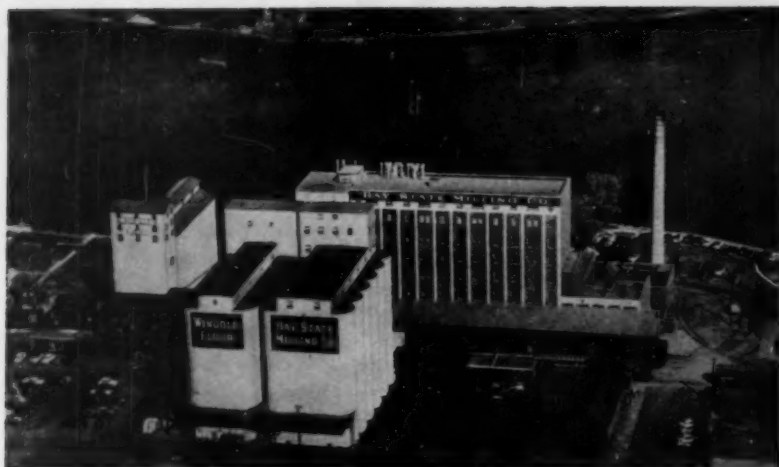
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